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AMRL-TR-66-110

METABOLISM and PHARMACOLOGY of FLUORINE-CONTAINING COMPOUNDS

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MIDWEST RESEARCH INSTITUTE

JULY 1966

AEROSPACE MEDICAL RESEARCH LABORATORIES
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The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

800 - September 1966 5-112

METABOLISM and PHARMACOLOGY of FLUORINE-CONTAINING COMPOUNDS

CHENG-CHUN LEE

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FOREWORD

This report was prepared by Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under USAF Contract No. AF 33(615)-2472, project 6302, "Toxic Hazards of Propellants and Material," and task 630202, "Pharmacology-Biochemistry." The work was administered under the direction of the Biomedical Laboratory, Aerospace Medical Research Laboratories, with Dr. Kenneth C. Back, Chief, Toxicology Branch, Toxic Hazards Division, acting as contract monitor.

This project was carried out in the Biological Sciences Division, under the direction of Dr. W. B. House, between 3 February 1965 and 1 June 1966. The experimental work was directly supervised by Dr. Cheng-Chun Lee, Senior Pharmacologist, with the technical assistance of Mr. William B. Jacobs, Mrs. Alma M. Landes and Mr. Paul M. Lambert. Dr. Thomas R. Castles and Mr. Chester R. Crawford, pharmacologists, offered valuable advice throughout the project. Drs. John W. Nebgen and Theodore S. Hermann, Senior Chemists, offered assistance for analytical analysis of the chemicals. The chamber, chemical induction system and supporting equipment were designed and fabricated under the direction of Mr. Fred J. Bergman.

This technical report has been reviewed and is approved.

WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory
Aerospace Medical Research Laboratories

ABSTRACT

Acute inhalation, intraperitoneal and/or oral toxicities of AMOX and TAMA were determined in rats and mice. IP absorption was studied in rats.

AMOX produced strong irritation resulting in pulmonary edema and hemorrhage after inhalation; and local denatured changes and accumulation of clear exudate after intraperitoneal injection. TAMA was less toxic on the weight basis.

Both AMOX and TAMA were quickly absorbed after IP injection. Fluoride increased quickly in the blood and concentrated in various tissues especially in the thyroid, bone and teeth. Most of the administered fluoride was excreted in the urine and a considerable amount was also recovered in the feces.

TABLE OF CONTENTS

ILLUSTRATIONS

TABLES

TABLES (Continued)

TABLES (Concluded)

SECTION I

INTRODUCTION

In recent years, despite the increasing interest in the fluorine-containing compounds used in rocket propellant systems, very little information concerning the pharmacological and metabolic properties of these compounds exists in the scientific literature. It is important to determine their toxicity and to obtain information on the fate of these compounds in physiological systems. This information is needed for diagnostic and therapeutic purposes and to establish guidelines for safety practices.

The biological effects of two fluorine-containing compounds, designated as AMOX and TAMA, are summarized. Acute inhalation, intraperitoneal and/or oral toxicity studies were performed on rats and mice. Experiments were designed to define possible mechanisms of toxicity. Quantitative and qualitative analytical methods were also devised to detect AMOX in air and fluoride in biological fluids and various tissues. These methods were used to study the absorption, distribution, and excretion patterns of both AMOX and TAMA in rats after intraperitoneal injection.

SECTION II

METHODS AND PROCEDURES

A. Experimental Animals

Disease-free inbred strains of albino rats (CFE) and mice (ICR) were obtained from Carworth Farms, Inc., New City, New York, and Southern Animal Farms, Prattville, Alabama, respectively. They were delivered via air express and were maintained in the laboratory quarters for at least 1 week to recover from the effects of shipment. The albino rabbits used for eye mucosa irritation studies were obtained locally. All animals were fed with commercial laboratory chow, and water was available at all times.

The rats or mice needed for one section of the studies, such as toxicity, clinical laboratory, or tissue fluoride distribution, were used from one shipment. The weights of animals at the time of experimentation were kept within a narrow range as much as possible.

B. Test Compounds

The two compounds that were studied are designated as AMOX and TAMA.

1. AMOX

AMOX was shipped and stored in a pressurized stainless steel cylinder (2.25-liter capacity) fitted with stainless steel valves. It is in a gaseous state under atmospheric pressure and at room temperature. Although we had expected the AMOX to be pure, we noted a slight brownish coloration due to NO_2 contamination. The intensity of the coloration indicated that the concentration of NO_2 was less than 1%.

The AMOX was studied in rats and mice after inhalation and intraperitoneal administration. For animal studies and for setting up a calibration curve, known quantities of AMOX were measured. A glass sample container was designed and constructed for transferring AMOX from the pressurized stainless steel cylinder. AMOX was reported to be stable in Pyrex® glass (ref 1). In these studies, pure AMOX might have slowly reacted with the glass to form SiF_4. The glass sample container used to transfer AMOX was first flushed with nitrogen and then with AMOX. When it was filled with AMOX, the inside glass surface was slowly etched and appeared to be cloudy. After further standing, the cloudiness inside the container became intense and white smoke steamed out of the container when the stopcock was open. It was necessary to evacuate and refill the sample container from which known quantities of AMOX were measured. AMOX, when diluted and mixed with air in the inhalation chamber, did not affect the chamber wall, which was made of Pyrex glass.

To avoid the degradation of AMOX, a stainless steel sample bomb of 150-ml capacity was constructed and used during the transfer of the material. A monel valve was welded to one end of the bomb. A Teflon® plug with a hole to accept a 22-gauge needle was tightly fitted into the outlet of the monel valve. A stainless ball valve was connected to the other end of the bomb. The outlet of this valve was fitted with a stainless steel socket; a stainless steel ball joint on the supply line from the reservoir containing AMOX was fitted into the stainless steel socket. The ball joint was lubricated with Kel-F® grease.

When AMOX was transferred from the reservoir, the sample bomb was connected to the supplying line through the ball joint and then evacuated with an aspirator. After the container had been filled with AMOX at a slight positive pressure, both the ball valve and the supply line valve were closed. The needle of a gas-tight syringe was then inserted through the hole of the Teflon plug at one end of the container, and the monel valve was opened for withdrawing measured amounts of AMOX.

AMOX for all animal studies was measured with a gas-tight syringe. The conversion of AMOX from volume to weight was made according to the following equations:

$$\text{Wt (g)} = \text{mole} \times \text{molecular weight of AMOX (87 g)} \qquad \text{(I)}$$

$$\text{Mole} = \frac{PV}{RT} \qquad \text{(II)}$$

where P is atmospheric pressure (1.0), V is volume (in liters), R is gas constant (0.082), and T is absolute temperature (room temperature = 273 + 25 K).

The presence of AMOX in the syringe or the leakage from the supply line could be detected by using filter paper wet with potassium iodide solution. The AMOX oxidized iodide and liberated free iodine which could be seen readily.

2. TAMA

TAMA is a liquid at room temperature and its shock sensitivity is comparable to that of nitroglycerine. To comply with ICC shipping regulations TAMA is desensitized by dilution with Freon 113.

Because of the high toxicity of Freon 113 in mice as reported later and because of the immiscible property of Freon 113 with blood, it was necessary to find a vehicle for injection studies. Communications with W. G. May of Esso Research and Engineering Company, Linden, New Jersey, manufacturer of TAMA, suggested that it might be soluble in many oxygenated and halogenated solvents. Some tests on TAA, a very closely related compound with various solvents, are shown below.

Solvent	Results
Freon 113	Miscible
Methylene Chloride	Miscible
Chloroform	Miscible
Nitrobenzene	Miscible
Ethyl Acetate	Miscible
Ethyl Ether	Miscible
Propyl Ether	Miscible
N-Pentane	Not Miscible
Heptane	Not Miscible
Carbon Tetrachloride	Partially Miscible
Ethyl Alcohol	Miscible but decomposes TAA
Water	Immiscible but decomposes TAA
Pyridine	Ignites pure TAA

None of the listed solvents miscible with TAA, and most likely also miscible with TAMA, was acceptable as a vehicle for intravenous administration. Therefore, it was decided to study several commonly used solvents employed by the drug manufacturer for injection. Freon 113 was stripped carefully from TAMA, employing a specially designed all-glass and Teflon apparatus mounted behind a barricade. Polyethylene glycol 200 was not miscible with pure TAMA. TAMA did dissolve in dimethyl sulfoxide, but the clear solution started to change color immediately from light yellow to orange, indicating the decomposition of the TAMA molecule.

Since no relatively nontoxic solvent could be found that would be miscible with blood, the studies involving intravenous injection were excluded from the program. Pure TAMA has a very low vapor pressure (ref 2). This characteristic severely limited the possibility of performing an inhalation toxicity study. Since the compound was highly shock sensitive, the conventional methods for vaporization could not be accomplished. Therefore, the TAMA solution in Freon 113 was studied in rats by the intraperitoneal and oral routes.

Known quantities of TAMA stock solution were first filtered into a graduated cylinder. Sufficient amounts of Freon 113 were used to wash the funnel and filter paper. The TAMA solution was made up to a known volume. This filtered TAMA solution was used to prepare various concentrations of working solutions. All doses were administered at a volume of 0.2 ml/100 g body weight. The working solutions of TAMA were freshly prepared from the stock solution each time prior to using.

C. Analytical Methods

1. AMOX in Chamber Air

A continuous infrared scanning technique was found to be excellent for determining qualitatively and quantitatively the presence of AMOX in air. The Model 137 NaCl Infracord® spectrophotometer was employed to scan AMOX. As shown in figure 1, AMOX had an intensive absorption band at 11.3 μ, which was chosen for measurement. Other absorption bands for AMOX were not sufficiently strong to measure at low concentrations.

2. Calibration Curve for AMOX

The volume of the 1-meter cell of the Model 137 NaCl Infracord spectrophotometer was 950 ml. The cell was evacuated with an aspirator and known quantities of AMOX were injected into the cell. Spectrophotograms were recorded accordingly.

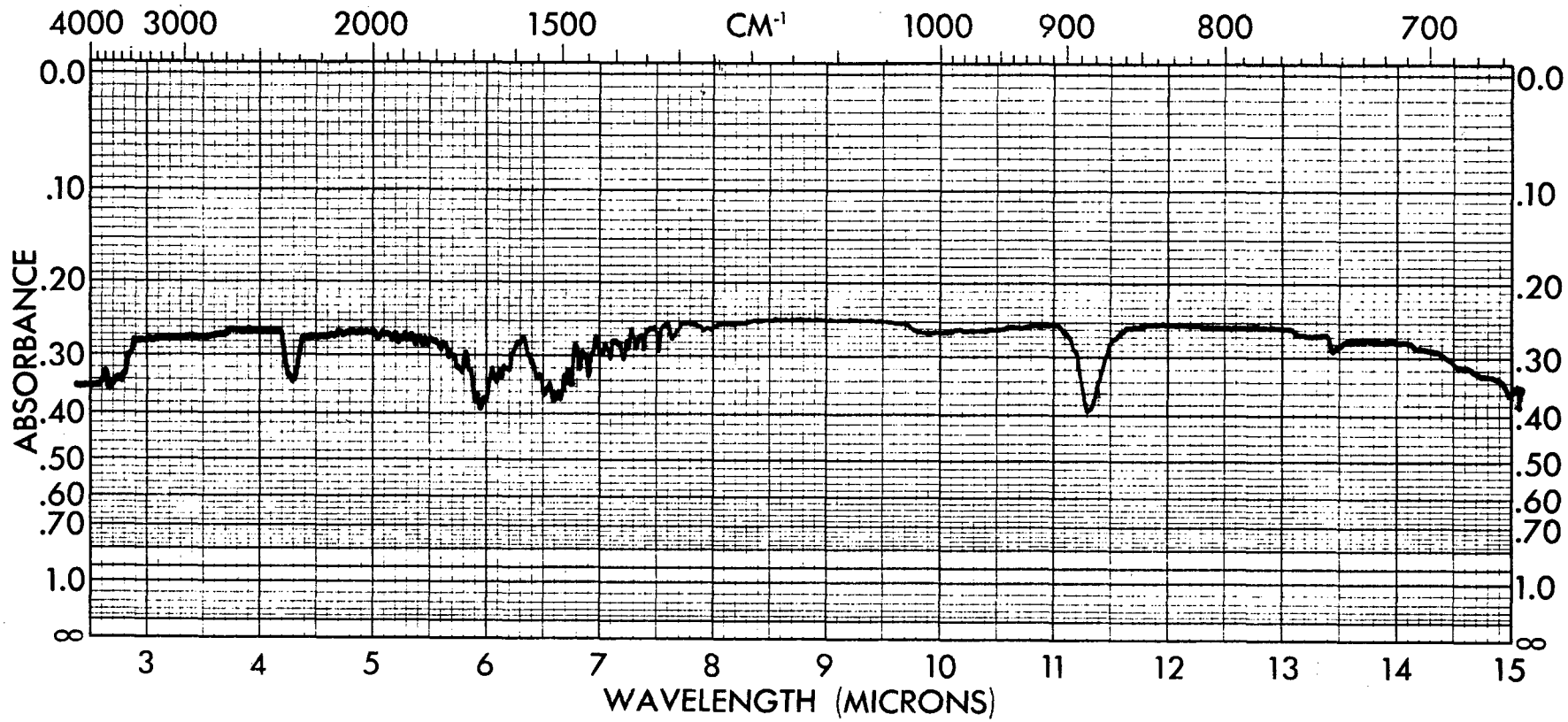


Figure 1 - Spectrophotogram of AMOX

The absorbance of AMOX at 11.3 μ was proportional to its concentration. The values of measured absorbance (A) were plotted against 10 ppm to 100 ppm of AMOX (C) and are shown in figure 2. The method of least squares was used to find the best fit. The equation for this fitted curve was:

$$\log A = - 2.808 + 1.535 \log C \qquad \text{(III)}$$

The estimated error was calculated (ref 3) to be ± 10%. From the fitted curve (figure 2), (C) of AMOX in any air sample could be readily determined from (A).

Determining the value of (A) required three measurements on the infrared paper. I_a was measured as the absorbance of the "flat line" across the top of the absorption band. I_b was the measured minimum absorbance. The difference, Δ , between these values in absorbance units multiplied by the "width" (the separation between absorption extremes in 0.1 μ paper units) gave the value of (A) as plotted on the calibration curve in figure 2. This value was twice the area of the absorption "triangle" which was a measure of the relative intensity.

The measurement was quite accurate when concentrations of AMOX were less than 100 ppm, and reasonably accurate if concentrations were between 100 and 300 ppm.

3. Fluoride Concentration in Tissues

Based on the general knowledge of the chemistry of fluorine-containing compounds, like AMOX and TAMA, they were expected to be highly reactive in a biological system. The detection and identification of the parent compounds and their metabolites in various tissues and excreta following administration to animals were not practical because of their high decomposition rate. However, methods were devised to determine quantitatively the total fluoride in biological materials.

The color reaction with zircono-alizarin (ref 4) as modified by Kornakova (refs 5 and 6) was found to be satisfactory for the determination of total fluoride in various biological materials, including blood, urine, soft tissues, bones, and teeth. The analytical procedure is described as follows:

a. Indicator solution: Twenty-three mg of alizarin sodium monosulfonate and 13 mg of zirconyl chloride ($ZrOCl_2$) were dissolved separately in demineralized water. The solution was mixed and made up to 1 liter with demineralized water. This indicator solution contained 1.5 x 10^{-4} moles/liter of combined components (Zr^{4+} and Na alizarinsulfonate = 1:1).

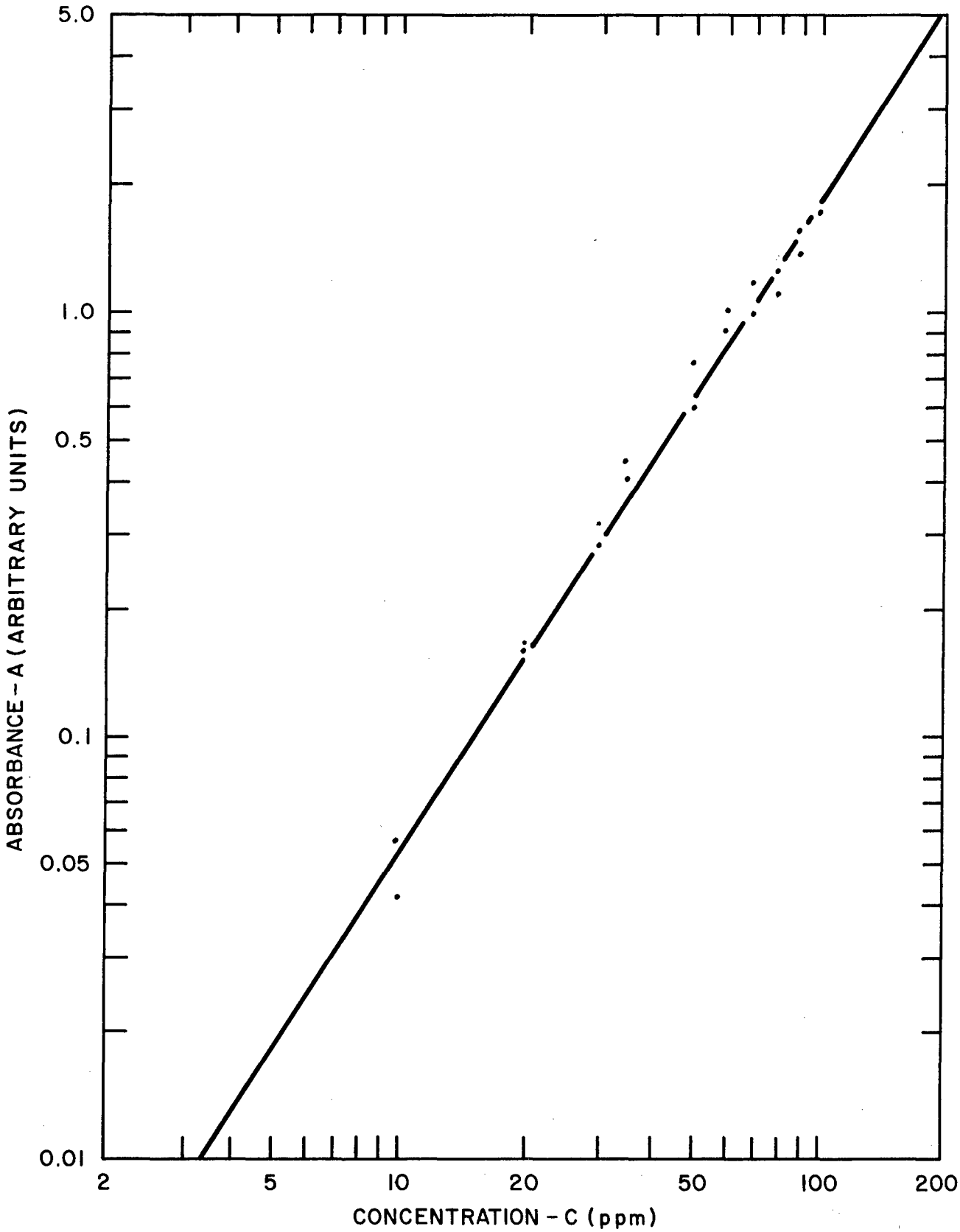


Figure 2 - Calibration Curve for AMOX

This indicator solution was originally used for samples containing 0.05 to 0.5 mg of fluoride. The standard curve was also extended to assay solutions containing 0.25 to 2.0 mg of fluoride. The concentration of the "strengthened" indicator solution was increased fourfold accordingly. These indicator solutions were freshly prepared and kept in the dark for the duration of usage.

b. Procedure for standard curve: The standard solvent was prepared according to Kornakova (ref 5). A volume of 0.5 ml of concentrated HCl and 25 ml of demineralized water was added to 3 ml of a saturated sodium carbonate solution. The pH of this mixture was adjusted to 7.6 to 7.8 with 4 N HCl. After the addition of 3.5 ml of 20% aqueous zinc acetate, the solution was heated in water bath at 50 C for 3 min. This solvent mixture was allowed to stand for 1 to 2 days before using and was stable for 1 week. The procedure for the determination of standard solutions containing various amounts of fluoride (as NaF) was as follows:

(1) A volume of 0.15 ml of freshly filtered standard solvent was added to each of a series of test tubes.

(2) Appropriate amounts of a fluoride solution (0.1 to 1.0 µg/ml) were added to each tube in duplicates containing 0.0, 0.025, 0.05, 0.10, and 0.20 µg or 0.0, 0.25, 0.50, 1.0, and 2.0 µg of F^-. The volume in each tube did not exceed 3 ml.

(3) A volume of 0.4 ml of 6 N HCl was added to each tube.

(4) Sufficient amounts of demineralized water were added to each tube to provide a total volume of 4 ml.

(5) A volume of 1.0 ml of the indicator solution was added. The original indicator solution was used for a standard curve of 0 to 0.2 µg of fluoride, whereas the "strengthened" indicator solution was used for a standard curve of 0 to 2.0 µg of fluoride. The color was originally developed in 20 min at room temperature. As the concentration of the indicator complex was increased fourfold, the reaction mixtures were incubated in a water bath at 37 C for 1 hr to complete the color development. These color solutions were stable for more than 24 hr.

(6) The color solutions were read at 540 µ in a Coleman Junior Spectrophotometer with the solution containing the highest amount of fluoride set at 100% transmittance (T). As shown in the upper half of table 1, the percent was directly proportional to the amount of fluoride. All results were reproducible.

TABLE 1

PERCENT TRANSMITTANCE IN SAMPLES CONTAINING VARIOUS AMOUNTS OF FLUORIDE

Amount of Fluoride (µg)				
0.0	0.25	0.5	1.0	2.0
Solution containing 2.0 µg set at 100 % T.				
84.0	85.8	88.0	92.0	100.0
83.8	85.5	87.8	91.8	100.0
83.8	84.5	88.0	92.2	100.0
84.0	86.0	88.2	92.8	100.0
83.8	86.0	88.8	92.5	100.0
84.2	86.0	88.0	92.0	100.0
84.5	86.0	88.5	92.2	100.0
84.0	86.2	88.2	90.8	100.0
84.0 ± 0.6*	85.7 ± 0.2	88.2 ± 0.1	92.0 ± 0.2	100.0
Distilled water blank set at 100 % T.				
70.2	72.0	75.0	78.8	89.2
70.0	72.2	74.8	79.0	89.0
69.8	72.5	74.5	79.2	89.0
70.0	72.0	74.0	78.0	87.8
70.0	72.0	73.8	77.8	88.8
70.0	72.2	74.0	78.5	89.2
69.8	73.0	75.0	78.2	89.0
69.8	72.8	75.8	78.5	89.5
70.0	73.0	75.5	79.0	89.5
72.0	73.8	76.0	80.5	89.0
70.2 ± 0.2*	72.6 ± 0.2	75.0 ± 0.2	78.9 ± 0.2	89.0 ± 0.1

* Average ± SE

TABLE 1 (Concluded)

Amount of Fluoride (μg)				
0.0	0.025	0.05	0.10	0.20
Distilled water blank set at 100% T.				
80.2	81.5	83.2	86.2	92.0
80.2	81.2	83.8	86.8	92.2
80.0	81.2	83.5	85.8	91.8
80.5	81.6	84.2	86.0	90.5
80.2	81.2	82.8	86.2	93.2
80.8	81.5	83.6	87.0	92.2
80.8	81.8	83.2	87.2	90.0
80.5	81.5	83.8	86.2	91.0
80.2	81.8	83.8	86.0	92.0
80.4 ± 0.1*	81.4 ± 0.1	83.5 ± 0.1	86.4 ± 0.2	91.6 ± 0.3

* Average ± SE

The color solutions were also read with the demineralized water blank set at 100% T. This permitted one to read the percent transmittance between 60 and 90%. The results are presented in the lower half of table 1. The readings were reproducible without appreciable variation. For all subsequent determinations, the color solutions for standards, as well as for tissues, were read with a demineralized water blank set at 100% T.

Various interference substances including Na^{+}, K^{+}, Mg^{+2}, Ca^{+2}, Cr^{+5}, Fe^{+2}, Al^{+3}, Cl^{-}, Br^{-}, I^{-}, PO_4^{-3}, SO_4^{-2} in amounts of 50 µg or more were added to the fluoride standard solution. With the exception of Cr^{+5}, none were found to have any effect on the assay results. Determination of various levels of fluoride in standard solutions (as NaF) were also performed according to the procedure as described below for soft tissues, including the step of ashing. The results were the same as those obtained with the standard procedure.

The shape and slope of the standard curves varied slightly during day to day determinations. A standard curve was always prepared along with each set of unknown samples.

c. Procedure for soft tissues: The total fluoride concentrations were determined in various soft tissues and excreta. All soft tissues were trimmed free of fat and connective tissues and the wet weight of each tissue was recorded. The total excretion of feces was first freeze-dried and ground with a glass mortar. A portion of the powdered feces was used for assay. The procedure for total fluoride determination in tissues was as follows:

(1) The samples (less than 1 ml of blood or urine, less than 1 g of tissues or the entire organ) were placed separately in platinum crucibles.

(2) A volume of 0.3 ml of a saturated sodium carbonate solution was added. Five-tenths ml had to be added when the sample contained large amounts of fluoride. In this case, 0.25 ml of the standard solvent was used for the standard curve.

(3) All samples were freeze-dried.

(4) Each sample was converted to ash by heating in covered crucibles at 750 C for 2 hr.

(5) The ashed sample was transferred and rinsed with a total of 2 ml of demineralized hot water into a test tube.

(6) The pH was adjusted to 7.6 to 7.8 with 2 N HCl, and the volume of all samples was kept constant. Care had to be taken not to run the pH to the acid side.

(7) A volume of 0.3 ml of 20% zinc acetate solution was added, and the mixture was heated in a water bath at 50 C for 3 min.

(8) An additional 0.4 ml of the zinc acetate solution was added, and the mixture was again heated in a water bath at 50 C for 3 min.

(9) All samples were cooled and centrifuged for 10 min at 2,400 rpm.

(10) The supernatant was filtered into a clean tube.

(11) The precipitate was washed twice with 0.5 ml portions of demineralized water. A larger volume of water was used when the sample contained a large amount of fluoride. After centrifugation, the supernatant was filtered into the original filtrate. The entire amount of the filtrate or an aliquot was used for the subsequent steps.

(12) A volume of 0.4 ml of 6 N HCl was added.

(13) A sufficient amount of demineralized water was added to make a final volume of 4 ml.

(14) The color was developed as described for standard fluoride solutions.

(15) The amount of fluoride in each sample was determined by reading the percent T on the standard curve.

d. Procedure for bone and teeth: The femur was trimmed free of soft tissues. The femur or both the upper and lower incisors were freeze-dried. The teeth were ground with a glass mortar. Each sample was weighed and digested in 5 ml of 3 N NaOH at 70 C on a hot plate with continuous magnetic stirring for 30 min. A final hydrolysate containing fine particles was obtained. Demineralized water was added to make a final volume of 10 ml. An aliquot of 0.1 ml was placed in a platinum crucible. The sample was ashed, and the amount of fluoride determined according to the procedure described above for soft tissues.

e. Recoveries from blood and liver: Various amounts of fluoride as NaF were added to heparinized rat blood and the total fluoride was determined. Recoveries of added fluoride were satisfactory. As shown in table 2, the recoveries averaged 96.6, 94.6 and 96.0% when 0.25, 0.50 and 1.00 µg of fluoride were added to 0.5 ml of heparinized rat blood, respectively. However, the average recovery for 0.1 µg of fluoride added to the blood was slightly lower (90.0%). Heparin did not affect the result.

TABLE 2

RECOVERIES OF FLUORIDE ADDED TO 0.5 ML BLOOD SAMPLES

Fluoride Concentrations (µg)[a]	Fluoride Added (µg)	Fluoride Recovered (µg)	Recovery of Added Fluoride (%)
0.46 (2)	0.10	0.08	80
	0.10	0.07	70
0.43 (2)	0.10	0.09	90
	0.10	0.09	90
0.43 (2)	0.10	0.12	120
	0.10	0.09	90
		Average ± SE	90.0 ± 6.8
0.47 (2)	0.25	0.24	96
	0.25	0.23	92
0.49 (2)	0.25	0.23	92
	0.25	0.23	92
0.41 (3)	0.25	0.26	104
	0.25	0.24	96
	0.25	0.26	104
		Average ± SE	96.6 ± 2.0
0.47 (2)	0.50	0.46	92
	0.50	0.48	96
0.49 (4)	0.50	0.52	104
	0.50	0.45	90
	0.50	0.47	94
	0.50	0.46	92
		Average ± SE	94.6 ± 2.0
0.46 (3)	1.00	1.01	101
	1.00	0.96	96
	1.00	0.94	94
0.43 (2)	1.00	0.95	95
	1.00	0.94	94
		Average ± SE	96.0 ± 1.3

a/ Number of trials shown in parentheses.

The amount of sample had no effect on the assay results so long as the total fluoride content did not exceed 2 μg, which is the upper limit of the standard curve. Fluoride content in various amounts of blood was assayed. The results are presented in table 3. The amounts of fluoride in 0.1, 0.2, 0.4, and 0.8 ml of blood averaged 0.07, 0.17, 0.35, and 0.70 μg, respectively.

Similarly, when 0.25, 0.50, and 1.00 μg of fluoride were added to liver samples, the average recoveries were 98.0, 96.2, and 97.6%, respectively (table 4). On the other hand, the recovery for 0.10 μg of fluoride added to the liver was also lower (86.6%).

f. Recoveries from bone: For each series of studies, two femurs of a control rat were digested in 10 ml of 3 N NaOH containing 300 μg of fluoride (as NaF). The hydrolysate was used as the control sample. The fluoride was added to adjust the amount of fluoride in the final assay sample to about 0.6 μg. One ml of this hydrolysate was added to 3 ml of demineralized water containing 0, 10.0, 20.0 or 30.0 μg of fluoride. These three levels of fluoride added 0.25, 0.50, and 0.75 μg of fluoride to the final assay sample, respectively. All samples were likewise heated at 70 C on a hot plate for 30 min as described in the section on Procedure for Bone and Teeth. An aliquot of 0.4 ml of each sample was ashed and determined for total fluoride.

The recoveries of fluoride added to bone hydrolysate were satisfactory. As shown in table 5, the recoveries averaged 92.3, 87.0, and 97.0% when 0.25, 0.50, and 0.75 μg of fluoride, respectively, were added to the bone hydrolysate containing about 0.60 μg of fluoride.

D. Inhalation Chamber and Sampling Equipment for AMOX

The inhalation studies were conducted in a specially built chamber. The materials used in its construction and all auxiliary equipment that came in contact with the test compounds were limited to stainless steel (300 series), glass, and Teflon. All surfaces that came in contact with the concentrated test compounds were carefully degreased.

A detailed design of the constructed chamber is shown in figure 3. The chamber was cylindrical in shape, 16 in. in diameter, and 24 in. in height. The walls of the chamber, except for the service collar, consisted of Pyrex glass. The service collar with its inlet and exhaust piping was constructed of stainless steel. The top and bottom glass sections, where they came in contact with the stainless steel collar, were gasketed with Teflon. The animals were supported on a removable stainless steel rack consisting of two floor levels.

TABLE 3

FLUORIDE CONCENTRATIONS (μg/SAMPLE)
IN VARIOUS AMOUNTS OF BLOOD

	Amount of Blood (ml)			
Rat	0.1	0.2	0.4	0.8
1	0.08	0.17	0.33	0.73
2	0.06	0.16	0.34	0.69
3	0.08	0.19	0.38	0.75
4	0.07	0.19	0.39	0.77
5	0.06	0.15	0.31	0.66
Average ± SE	0.07 ± 0.004	0.17 ± 0.005	0.35 ± 0.015	0.70 ± 0.020

TABLE 4

RECOVERIES OF FLUORIDE ADDED TO LIVER SAMPLES

Liver Used (g)	Concentration of Fluoride (µg/g)[a]	Fluoride Added (µg)	Fluoride Recovered (µg)	Recovery of Added Fluoride (%)
0.2331	1.45 (2)	0.10	0.08	80
0.3611		0.10	0.09	90
0.3330		0.10	0.09	90
			Average ± SE	86.6 ± 3.3
0.5888	1.87 (2)	0.25	0.23	92
0.4886		0.25	0.28	112
0.4509		0.25	0.23	92
0.2763	1.47 (2)	0.25	0.24	96
0.3165		0.25	0.25	100
0.3291		0.25	0.24	96
			Average ± SE	98.0 ± 3.0
0.3460	1.47 (2)	0.50	0.48	96
0.3821		0.50	0.47	94
0.2907		0.50	0.46	91
0.4467	1.87 (2)	0.50	0.49	98
0.4282		0.50	0.50	100
0.4277		0.50	0.49	98
			Average ± SE	96.2 ± 1.3
0.4408	1.45 (2)	1.00	0.98	98
0.3466		1.00	0.97	97
0.3691		1.00	0.98	98
			Average ± SE	97.6 ± 0.4

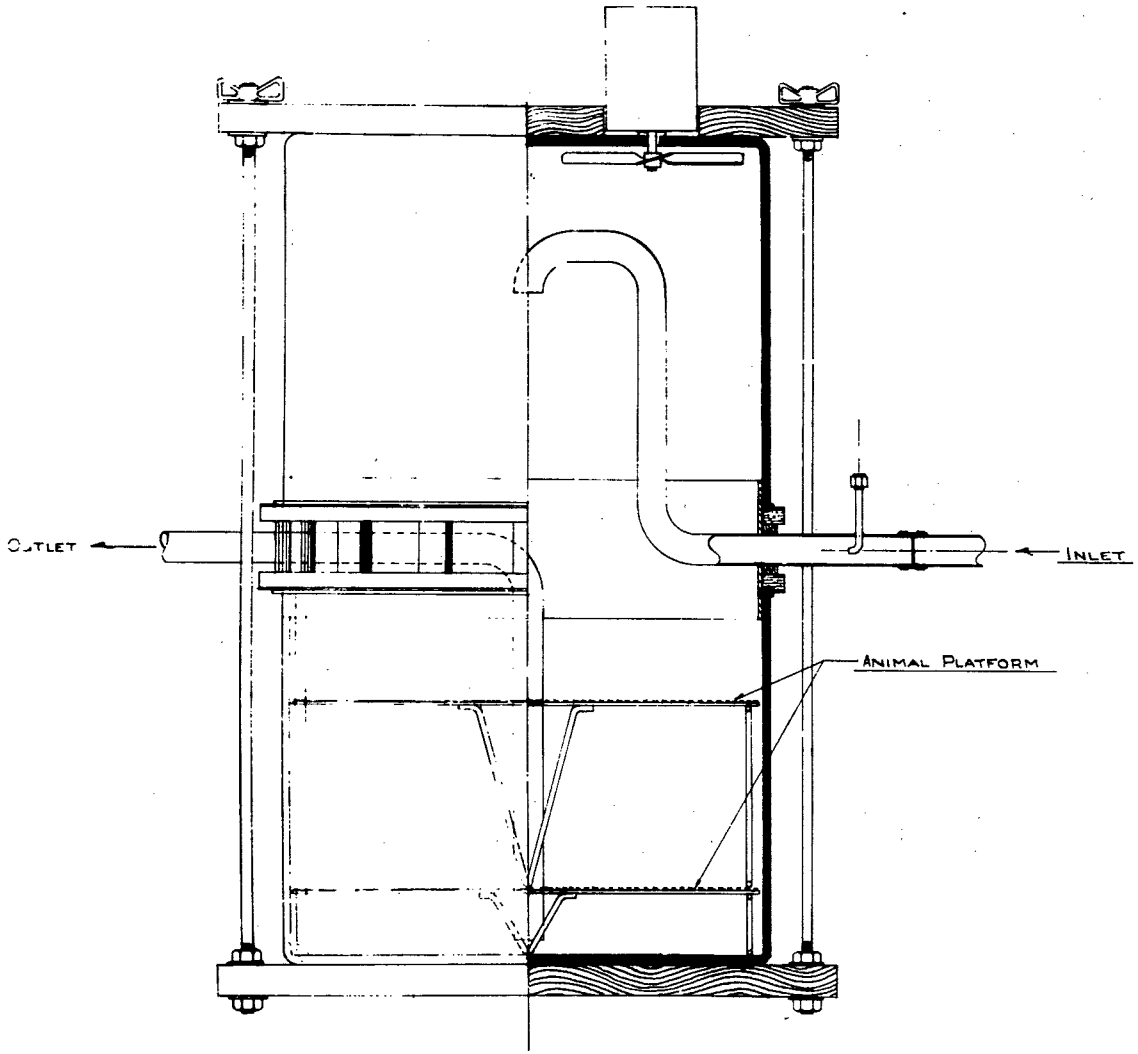
a/ Number of trials shown in parentheses.

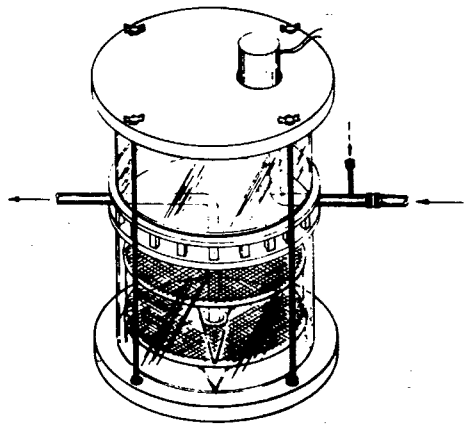
TABLE 5

RECOVERIES OF FLUORIDE ADDED TO FEMUR HYDROLYSATES

Fluoride Content (μg)[a]	Fluoride Added (μg)	Fluoride Recovered (μg)	Recovery of Added Fluoride (%)
0.56 (3)	0.25	0.23	92.0
	0.25	0.22	88.0
	0.25	0.24	96.0
0.65 (3)	0.25	0.22	88.0
	0.25	0.23	92.0
	0.25	0.26	104.0
		Average ± SE	92.3 ± 2.5
0.56 (3)	0.50	0.42	84.0
	0.50	0.48	96.0
	0.50	0.44	88.0
0.65 (3)	0.50	0.44	88.0
	0.50	0.36	72.0
	0.50	0.47	94.0
		Average ± SE	87.0 ± 3.5
0.56 (3)	0.75	0.82	109.0
	0.75	0.72	96.0
	0.75	0.70	93.0
0.65 (3)	0.75	0.77	103.0
	0.75	0.67	89.0
	0.75	0.69	92.0
		Average ± SE	97.0 ± 3.1

a/ Number of trials shown in parentheses.





ISOMETRIC VIEW OF CHAMBER
NO SCALE

Figure 3 - Inhalation Chamber

The chamber was operated on a continuous air stream utilizing variable speed blowers to control the air volume. The intake air was supplied from outside the room in which the inhalation chamber was located. The incoming air stream carrying the test compound was kept in a constant state of turbulence through the use of a fan placed at the top of the chamber. The exhaust duct had its intake extended below the lower animal support platform.

The induction system consisted of test compound storage, valves, and lines. The storage pressurized cylinder with its primary reducing valves was situated outside of the building. The AMOX was used directly from the shipping containers and was delivered through the stainless steel lines to the hood containing the chamber. Inside the hood, the AMOX was delivered to the induction panel through a micrometer to an expansion chamber and was introduced into the common air stream pipe by a jet positioned in the middle of the duct and pointing toward the plenum.

The exhaust system was devised to allow the continuous sampling of all the effluent gas from the chamber. The exhaust tube was built to accommodate an infrared gas cell of the Model 137 NaCl Infracord spectrophotometer. By adjusting the micrometer of the induction system, a desired concentration of AMOX in the chamber air could be obtained and monitored with the Infracord. The exhaust from the chamber was passed through a charcoal packed tower and exhausted through stacks to the outside of the building.

E. Clinical Laboratory Method

1. Hematology

a. Bleeding time: Duke's method (ref 7) was used by cutting the tip of the rat's tail and noting the time interval between the appearance of the first drop of blood and the removal of the last drop.

b. Coagulation time: Sabraze's capillary tube method (ref 8) was used by noting the time between the blood filling of the capillary tube and the appearance of fibrin thread at the broken ends.

c. Total erythrocyte and leukocyte counts: The method of Brecher et al (ref 9) was used by means of a Coulter Electronic Particle Counter®, employing a 100 μ aperture and correcting for coincident passage.

d. Differential leukocyte counts: Wright's stain was used.

e. Hematocrit: Hematocrit was determined in capillary tubes using a microcapillary centrifuge.

f. Hemoglobin: Hemoglobin was measured by the method of Crosby et al (ref 10) as cyanmethemoglobin.

g. Methemoglobin: Methemoglobin was measured by the spectrophotometric method of Hainline (ref 11).

h. Mean values for corpuscular volume (MCV), corpuscular hemoglobin (MCHb) and corpuscular hemoglobin concentration (MCHbC): These were calculated from appropriate data.

2. Functional Tests

a. Serum glutamic-oxaloacetic transaminase (SGOT): Oxaloacetate released by this enzyme was measured colorimetrically (ref 12).

b. Serum glutamic-pyruvic transaminase (SGPT): Pyruvate released by this enzyme was measured colorimetrically (ref 12).

SECTION III

RESULTS

A. AMOX

1. Acute Inhalation Toxicities

a. Exposure for 4 hr: Male rats and mice were exposed to various concentrations of AMOX for 4 hr. At the end of the exposure, all surviving animals were removed from the inhalation chamber and placed in cages as groups. Food and water were provided ad libitum. The number of deaths was recorded at the end of the exposure period and at the end of 1 day and 7 days after the beginning of exposure. The median lethal concentrations (LC_{50}) of AMOX and its 95% confidence limits were calculated using probit analysis of response against log doses as described by Finney (ref 13). The analysis was programmed on an IBM 1710 computer.

The concentrations of AMOX in chamber air for 4 hr are summarized in table 6. The chamber concentration of AMOX reached equilibrium within 5 min after closure of the chamber and was maintained relatively constant throughout each experiment.

The LC_{50}'s (4-hr exposure) for rats and mice are presented in table 7. The LC_{50} for male rats was 24.2 ppm at the end of 7 days. Most of the deaths occurred during the first 24 hr. Four of 15 rats exposed to the highest concentration of AMOX (35.0 ppm) died before the end of the 4-hr exposure period.

TABLE 6

CONCENTRATIONS OF AMOX (ppm) IN CHAMBER AIR FOR 4 HOURS

Concentration of AMOX (ppm)

Expt.	Animal Exposed	Avg. Conc.	Time After Inhalation Chamber was Closed										
			5 (Min)	10 (Min)	15 (Min)	30 (Min)	1 (Hr)	1.5 (Hr)	2.0 (Hr)	2.5 (Hr)	3.0 (Hr)	3.5 (Hr)	4.0 (Hr)
4	Rats	12.4	18	17	15	14	12	12	13	13	11	11	10
7	Rats	15.1	12	9	9	9	5	22	22	15	20	20	18
6	Rats	24.8	14	21	26	26	26	26	26	23	26	26	26
8	Rats	28.9	25	30	30	31	28	30	32	28	31	26	26
11	Rats	33.5	31	31	32	34	34	33	30	30	32	35	32
5	Rats	35.0		58	34	31	37	34	32	34	34	33	34
12	Mice	9.3	9	11	10	10	10	9	10	9	10	8	8
9	Mice	15.4	14	14	15	14	15	16	16	16	16	17	15
13	Mice	18.9	18	19	18	20	18	21	20	19	18	19	18
9	Mice	22.8	22	23	23	23	24	22	22	23	23	22	24
10	Mice	27.1	18	20	25	28	30	27	26	28	26	28	30

TABLE 7

MEDIAN LETHAL CONCENTRATIONS OF AMOX FOR RATS AND MICE AFTER EXPOSURE FOR 4 HOURS

Rats, Male, 175 - 275 g

	AMOX Concentration in Chamber (ppm)						LC_{50} (ppm) (95% CL)
	12.4	15.1	24.8	28.5	33.5	35.0	
4 hours	0/4[a]	0/16	0/16	0/16	0/16	4/15	
1 day	0/4	4/16	6/16	6/16	7/16	15/15	
7 days	0/4	4/16	7/16	6/16	9/16	15/15	24.2 (20.0 - 28.0)

Mice, Male, 22 - 30 g

	AMOX Concentration in Chamber (ppm)					LC_{50} (ppm) (95% CL)
	9.3	15.4	18.9	22.8	27.1	
4 hours	0/16	0/16	0/16	0/16	5/16	
1 day	0/16	4/16	7/16	10/16	15/16	19.8 (17.8 - 21.7)
7 days	0/16	5/16	8/16	16/16	16/16	17.5 (15.9 - 18.0)

a/ Number of deaths/number of animals exposed.

The number of deaths at the end of 1 day after exposure to the various concentrations were distributed in such a way that the LC_{50} could not be calculated correctly.

AMOX was more toxic to mice than to rats. The LC_{50} (4-hr exposure) for male mice was 19.8 and 17.5 ppm at the end of 1 day and 7 days, respectively. Five of 16 mice exposed to the highest concentration of AMOX (27.1 ppm) died before the end of exposure period.

b. Exposure for 15, 30, or 60 min: Groups of male rats were exposed to various concentrations of AMOX for 15, 30, or 60 min. The concentrations of AMOX in the chamber and the number of deaths recorded at the end of 4 hr, 1 day, and 7 days after exposure for 15, 30, or 60 min are summarized in tables 8, 9, and 10, respectively. The desired concentration of AMOX was reached quickly after closure of the chamber and was maintained relatively constant throughout each experiment. Most of the rats that died succumbed within 1 day. The LC_{50}'s for 15, 30, and 60 min exposures were 202 to 240 ppm, 119 to 149 ppm, and 87 to 104 ppm, respectively. Exact LC_{50}'s could not be calculated because of the distribution of the deaths at the various concentrations of AMOX. Furthermore, chamber concentrations of AMOX could not be precisely monitored with our equipment at these higher concentrations.

c. Symptoms: When rats and mice were placed in the inhalation chamber, expecially at higher concentrations, all animals showed increased activity and excitation for various lengths of time. Then they became depressed and motionless throughout the remaining time of exposure. Prior to death, the rats amd mice had great difficulty in breathing. This symptom was followed by convulsions and death while they were still in the inhalation chamber. The animals that survived the exposure of AMOX showed severe depression, loss of muscle tone, and weakness.

d. Pulmonary lesions: The lungs of all the rats and mice that died during or after AMOX exposure showed extensive hemorrhages. The size of the lungs was grossly larger than those of untreated animals; the lung tissue, including trachea, was filled with bloody exudates. The lungs of several treated animals were ligated at the hilum and removed. After blotting with filter paper, the wet weight of each lung (including the trapped fluid) was determined. Lung weights were increased 368 and 256% over those of control rats and mice, respectively, as presented in table 11. The lungs of treated animals as well as controls were also photographed and are shown in figure 4.

At several intervals up to 1 month after exposure, surviving animals from most groups, especially those exposed to higher concentrations of AMOX, were sacrificed. Their lungs showed various stages of regeneration. The size of lung varied depending upon the concentration of AMOX to which they had been exposed and the time they had been removed from exposure.

TABLE 8

CONCENTRATIONS OF AMOX (ppm) IN CHAMBER AIR AND MORTALITY OF RATS AFTER EXPOSURE FOR 15 MINUTES

Expt. No.[a]	Concentration of AMOX (ppm)				Number of Deaths		
	Average Concentration	Minutes After Rats in Chamber					
		5	10	15	4 Hr	1 Day	7 Days
19	52	48	49	60	0	0	0
22	102	105	101	99	0	0	0
32	154	148	152	162	0	0	0
40	202	200	210	195	0	0	0
43	215	216	214	214	4	4	8
42	240	260	235	225	11	11	12
36	380	380	380	380	12	12	12

TABLE 9

CONCENTRATIONS OF AMOX (ppm) IN CHAMBER AIR AND MORTALITY OF RATS AFTER EXPOSURE FOR 30 MINUTES

Expt. No.[a]	Concentration of AMOX (ppm)							Number of Deaths		
	Average Concentration	Minutes After Rats in Chamber								
		5	10	15	20	25	30	4 Hr	1 Day	7 Days
18	50	30	52	53	51	60	56	0	0	0
21	84	84	84	86	79	79	91	0	0	0
34	119	124	118	124	120	112	116	0	0	0
41	137	130	135	140	148	135	135	0	5	5
31	149	140	152	150	150	148	152	9	12	12

a/ Each group consisted of 12 male rats weighing between 180 and 250 g.

TABLE 10

CONCENTRATIONS OF AMOX (ppm) IN CHAMBER AIR AND MORTALITY OF RATS AFTER EXPOSURE FOR 60 MINUTES

Expt. No.[a]	Concentration of AMOX (ppm)							Number of Deaths		
	Average[b] Concentration	Minutes After Rats in Chamber						4 Hr	1 Day	7 Days
		5	10	15	30	45	60			
20	39	37	38	40	40	39	38	0	0	0
23	45	44	44	44	45	45	47	0	0	0
26	59	56	57	60	59	60	60	0	0	0
33	67	74	72	73	60	76	68	0	0	0
35	87	79	86	86	90	88	88	0	0	0
39	94	86	99	96	93	100	90	6	10	10
38	104	110	102	100	102	97	112	10	12	12
37	116	116	116	125	114	116	115	12	12	12

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a/ Each group consisted of 12 male rats weighing between 180 and 250 g.

b/ Concentrations at 5, 10 and 15 min were first averaged. This average concentration was used to obtain the over-all average concentration for the entire experiment.

TABLE 11

WET WEIGHT OF THE LUNG INCLUDING TRAPPED FLUID
OF ANIMALS EXPOSED TO AMOX

	Body Weight (g)	Lung Weight (g)	Lung Weight (g/kg)	Average (g/kg)
Rat-control	230	1.46	6.36	5.16
	260	1.23	4.73	
	292	1.42	4.86	
	305	1.43	4.70	
Rats-treated	234	4.16	18.00	18.98
	260	3.22	12.37	
	234	5.60	23.94	
	222	4.80	21.60	
Mouse-control	22	0.14	6.41	7.41
	25	0.19	7.59	
	24	0.18	7.64	
	23	0.18	7.98	
Mice-treated	23	0.50	21.91	18.45
	22	0.35	15.93	
	22	0.37	15.66	

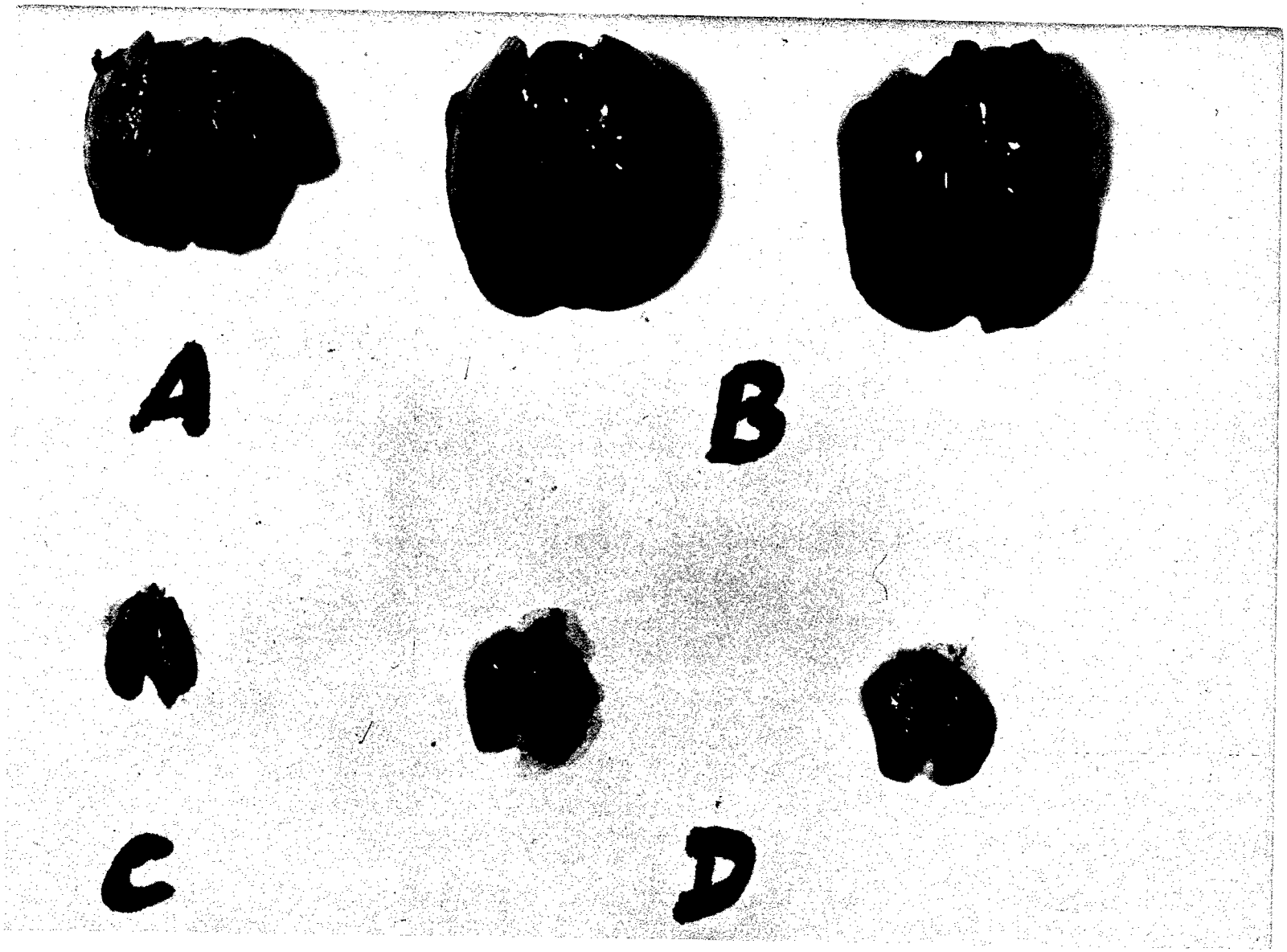

Figure 4 - The Lung from Untreated Animals and Animals Exposed to AMOX: (A) Untreated rat; (B) Treated rats; (C) Untreated mouse; (D) Treated mice.

The bloody exudates gradually disappeared and the color of the lung changed from a grayish to pinkish color. Animals that survived for 7 days following their removal from the exposure chamber recovered.

2. Acute Intraperitoneal Toxicities

AMOX was injected intraperitoneally to rats and mice by means of a gas-tight syringe at atmospheric pressure and room temperature. After injection, all animals were placed in cages as groups. Feed and water were available at all times. The number of deaths were recorded at the end of 1 day and 7 days.

a. Median lethal dose (LD_{50}): The LD_{50} was calculated similarly as for LC_{50}. The dose levels of AMOX and LD_{50} for rats and mice are summarized in table 12. The LD_{50} for female rats at the end of 7 days after injection was 10.8 ml/kg body weight. When converted into weight, the LD_{50} was 38.4 mg/kg body weight. Most of the deaths at the various dose levels occurred within 1 day after injection. The LD_{50} at the end of 1 day was about the same as that at the end of 7 days. After the first day, the surviving rats recovered from the toxic effects of AMOX.

The male rats were more resistant to the toxic effects of AMOX than were the females. The LD_{50} for male rats at the end of 7 days after injection was 14.5 ml(51.6 mg)/kg body weight, which was significantly higher than that for the females. Nearly all deaths occurred within the first day after injection. The LD_{50} value at the end of 1 day was about the same as that at the end of 7 days.

The LD_{50} for female mice at the end of 7 days after injection was 8.3 ml(29.5 mg)/kg body weight. At the end of 1 day, the LD_{50} was slightly higher, i.e., 10.6 ml(37.6 mg)/kg body weight. Male mice were slightly more resistant than the females to the toxic effects of AMOX. The LD_{50} values for the males were 11.8 ml(42.2 mg)/kg body weight at the end of 1 day and 9.2 ml(32.1 mg)/kg body weight at the end of 7 days.

b. Symptoms and gross pathology: Following administration of higher doses of AMOX, rats and mice showed various degrees of distress within 15 min. The observed symptoms included arched back, loss of abdominal muscle tone, labored and rapid respiration, ptosis, defecation, nonhuddling, weakness, and death.

When the abdominal cavity was examined, all rats showed extensive denatured changes, especially of the peritoneal membrane and fat around the kidneys. Large amounts of exudate were also found in the abdominal and thoracic cavities.

TABLE 12

DOSE LEVELS AND LD_{50} OF AMOX FOR RATS AND MICE AFTER INTRAPERITONEAL INJECTION

		Dose Level ml/kg / mg/kg						LD_{50} (95% CL) ml/kg	LD_{50} (95% CL) mg/kg
Rat:	Females, 200 - 280 g								
		5.0 / 17.8	7.5 / 26.7	10.0 / 35.6	12.5 / 44.5	15.0 / 53.4			
	1 day	0/20[a]	2/20	6/20	12/20	15/15		11.0 (10.1-12.0)	39.2 (36.0-42.9)
	7 days	1/20	2/20	7/20	12/20	15/15		10.8 (9.7-12.0)	38.4 (34.6-42.8)
	Males, 275 - 335 g								
		7.5 / 26.7	10.0 / 35.6	12.5 / 44.5	15.0 / 53.4	17.5 / 62.3	20.0 / 71.2		
	1 day	0/5	0/17	7/23	10/18	12/17	14/16	14.6 (13.5-15.8)	52.0 (48.2-56.1)
	7 days	0/5	1/17	7/23	10/18	12/17	14/16	14.5 (13.4-15.7)	51.6 (47.6-56.0)
Mice:	Females, 18 - 30 g								
		5.0 / 17.8	7.5 / 26.7	10.0 / 35.6	12.5 / 44.5	15.0 / 53.4			
	1 day	0/15[a]	2/15	7/15	10/15	6/6		10.6 (9.4-11.9)	37.6 (33.5-42.5)
	7 days	0/15	7/15	10/15	15/15	6/6		8.3 (7.3-9.2)	29.5 (25.9-32.8)
	Males, 20 - 35 g								
		7.5 / 26.7	10.0 / 35.6	12.5 / 44.5	15.0 / 53.4	17.5 / 62.3			
	1 day	0/12	0/14	11/14	13/14	6/6		11.8 (10.9-12.7)	42.2 (38.9-45.1)
	7 days	5/12	5/14	13/14	14/14	6/6		9.2 (7.4-10.1)	32.1 (26.4-36.1)

a/ Number of deaths/number of treated.

c. Pulmonary lesions: Since the inhalation of AMOX was found to cause various degrees of pulmonary lesions in rats, the effect of AMOX after intraperitoneal injection on edema formation was studied. Quantitative measurement of lung weight has been proven useful in the assessment of acute pulmonary edema formation (refs 14 and 15). A total of 24 rats was employed in this study. Six rats without treatment served as controls. The remaining 18 rats were given 15 ml(53.4 mg)/kg body weight of AMOX intraperitoneally. Six rats each were sacrificed under ether anesthesia at the end of 1, 2, and 3 days. The thorax of each rat was opened and the lung was ligated at the hilum. The entire lung was removed and blotted with filter paper. The wet weight of each lung, including trapped fluid, was obtained and the results are presented in table 13. The wet weight of the lung was significantly increased ($P < 0.01$) at the end of 1 day following intraperitoneal injection of AMOX; and thereafter, returned to the normal range of the control rats.

d. Effect of adrenal weight: The adrenals from control rats and from rats that were given 10 ml(35.6 mg)/kg of AMOX were removed and weighed. The results are summarized in table 14. The adrenals from rats at 4 hr, 1 day, and 7 days after AMOX administration were all significantly enlarged when compared with those of the control rats.

e. Acute intraperitoneal toxicity of HF: The toxicity of AMOX may be due to hydrofluoric acid (HF), a possible breakdown product of AMOX in the body. HF is highly reactive and would combine with electrolytes rapidly. Various doses of HF were injected intraperitoneally into female rats at a volume of 2 ml/kg body weight. A dose of 25 mg of HF/kg body weight killed all rats, whereas no rats died when a dose of 20 mg/kg was given. The toxicity of HF appeared to be similar to that of AMOX. Most rats died within the first few hours after injection, and clear exudate was found in the abdominal cavity.

3. Effect on Blood Pressure

The effect of AMOX on blood pressure and EKG was studied in male rats. Each rat was anesthetized with an intraperitoneal injection of 40 mg/kg of pentobarbital sodium. Blood pressure was recorded via the carotid artery with a Statham pressure transducer and the EKG via standard lead II with a Beckman (Offner Division) Dynograph®. One rat weighing 325 g was given AMOX intraperitoneally 15 ml(53.4 mg)/kg body weight. There was an immediate drop of blood pressure from 110 mm Hg to 70 mm Hg. This immediate drop was due to mechanical handling of the animal. The blood pressure partially recovered to 80 mm Hg in 40 min and dropped again consistently thereafter. When this rat died 90 min after AMOX administration, the blood pressure was down to 20 mm Hg. A second rat weighing 330 g was given AMOX intraperitoneally 22.5 ml(80.1 mg)/kg body weight. The blood pressure again dropped immediately from 120 mm Hg to 75 mm Hg and continued to decrease. It reached a low of 25 mm Hg in 160 min when the rat died. There was no immediate effect on EKG in either rat.

TABLE 13

WET WEIGHT OF LUNGS INCLUDING TRAPPED FLUID (g/kg BODY WEIGHT) AFTER INTRAPERITONEAL INJECTION OF AMOX TO RATS

	Time After Injection			
Untreated	4 Hr	1 Day	2 Days	3 Days
5.11	4.93	7.59	5.38	6.29
5.64	6.09	6.00	6.13	6.08
7.09	5.52	6.43	5.55	6.14
5.92	5.32	8.31	5.62	5.27
4.96	5.50	8.76	5.74	5.27
5.52	5.99	5.89	5.34	5.59
Mean				
5.70	5.56	7.16*	5.63	5.86

* Kramer's modification of Duncan's new multiple range test (ref 16): $P < 0.01$, Coefficient variability = 11.8%.

TABLE 14

WET WEIGHT OF ADRENALS (mg/kg BODY WEIGHT)
AFTER INTRAPERITONEAL INJECTION OF AMOX TO RATS

	Time After AMOX Administration			
Control	1 Hr	4 Hr	1 Day	7 Days
205	162	225	266	260
205	190	257	236	279
185	225	188	200	295
204	207	258	245	259
167	190	250	220	266
167	224	-	271	249
191	-	-	180	280
171	-	-	-	-
Average ± SE				
187 ± 5	200 ± 9*	236 ± 14*	231 ± 12*	270 ± 4*

* Student "t" test (ref 17): $P < 0.01$.

Another rat was likewise given air intraperitoneally 22.5 ml/kg body weight. There was an initial decrease in blood pressure of 50 mm Hg. The blood pressure recovered gradually to the level before administration of the air. When this rat was sacrificed 5.5 hr after air injection, the blood pressure remained at 125 mm Hg.

4. Effects on Clinical Laboratory Results

Each of four groups of eight male rats was given intraperitoneally 10.0 ml(35.6 mg)/kg body weight of AMOX. Three of the 32 rats died. This dose of AMOX was about two-thirds the LD_{50} and was employed for all absorption, excretion and distribution studies of fluoride. At the end of 1 and 4 hr, and 1 and 7 days, the bleeding time for each animal was determined. Immediately thereafter, each rat was anesthetized with ether. About 0.3 ml of blood was withdrawn by heart puncture for the determination of coagulation time. The remaining blood was removed from the abdominal aorta through a middle ventral incision and heparinized for hematology studies and determination of transaminases. Blood was also taken from a group of untreated rats and was used as control samples.

The results obtained from these rats are summarized in table 15. The laboratory clinical data after AMOX administration were compared with those obtained from control rats. Following AMOX administration, bleeding time increased significantly at 1 hr and decreased at 4 hr. The bleeding time increased again at 1 day and returned to nearly normal levels at 7 days. Clotting time of these rats was not significantly changed at 1 and 4 hr, or at 7 days. However, it was increased at 1 day. The reason for these inconsistent changes in both bleeding and clotting times after AMOX administration is not understood.

The red blood cell count was significantly increased 1 hr after AMOX administration, accompanied by an increase in hematocrit and hemoglobin concentration. Both the mean corpuscular volume and the mean corpuscular hemoglobin decreased, whereas the mean corpuscular hemoglobin concentration remained unchanged at this time. When these rats were sacrificed, the abdominal cavity contained a considerable amount (up to 4 ml) of clear exudates and showed extensive denatured changes, especially of the peritonium and fat around the kidneys. The increases in red blood cell count, hematocrit and hemoglobin concentration were mainly due to movement of body fluids into abdominal cavity.

The red blood cell count returned to approximate normal level at 4 hr after AMOX administration, and dropped significantly at 1 day. At this time, both the hematocrit and hemoglobin concentrations also significantly decreased whereas both the mean corpuscular volume and the mean corpuscular hemoglobin increased. These changes continued to exist 7 days after AMOX administration.

TABLE 15

CLINICAL LABORATORY DATA OF RATS AFTER INTRAPERITONEAL INJECTION OF AMOX

	Control	Time After AMOX Administration			
		1 Hr	4 Hr	1 Day	7 Days
No. of Animals	8	8	6	7	8
Body Weight (g)	185 - 220	190 - 215	160 - 205	185 - 205	150 - 175
Bleeding Time (sec)	199 ± 21	466 ± 90[a]	110 ± 18[b]	365 ± 20[b]	258 ± 33
Clotting Time (sec)	116 ± 22	80 ± 15	106 ± 15	211 ± 31[a]	107 ± 16
RBC (x 10^6)	5.85 ± 0.13	7.63 ± 0.16[b]	5.23 ± 0.27	3.78 ± 0.22[b]	4.44 ± 0.11[b]
WBC (x 10^3)	7.9 ± 0.5	7.9 ± 1.7	10.4 ± 1.6	9.8 ± 2.4	8.4 ± 1.1
Lymphocytes (%)	82 ± 4	75 ± 1	54 ± 7[b]	74 ± 3	86 ± 2
Neutrophils (%)	16 ± 4	20 ± 4	44 ± 6[b]	24 ± 3	12 ± 2
Eosinophils (%)	0	1	0	0	1
Basophils (%)	0	0	0	0	0
Monocytes (%)	2	4	2	2	1
Hematocrit (vol %)	43 ± 1	52 ± 1[b]	47 ± 1[a]	39 ± 1[a]	45 ± 1
Hemoglobin (g %)	13.3 ± 0.2	15.7 ± 0.5[b]	15.0 ± 0.6[a]	11.5 ± 0.3[b]	13.4 ± 0.3
MetHb (g %)	0	2.2 ± 0.12	0	0	0
MCV (μ^3)	73.8 ± 1.5	67.6 ± 0.9[b]	90.5 ± 1.6[b]	104.7 ± 5.2[b]	101.5 ± 3.5[b]
MCHb ($\mu\mu g$)	22.5 ± 0.4	20.4 ± 0.2[b]	29.8 ± 1.9[b]	29.1 ± 0.3[b]	29.5 ± 0.3[b]
MCHbC (%)	30.8 ± 0.2	30.2 ± 0.4	28.8 ± 0.9	31.0 ± 1.8	30.1 ± 1.2
GOT (S F Units)	99 ± 14	258 ± 6[b]	216 ± 70	195 ± 31[a]	129 ± 8
GPT (S F Units)	38 ± 2	93 ± 12[b]	87 ± 10[b]	79 ± 10[b]	44 ± 1[b]

Student "t" test (ref 17): a/ $P < 0.05$, b/ $P < 0.01$

The AMOX also caused the formation of methemoglobin, averaging 2.2 g % (about 14% of the total hemoglobin), 1 hr after injection.

The total white blood cell count was not appreciably altered following the administration of AMOX. However, 4 hr after AMOX injection, the percent of the neutrophils significantly increased, accompanied by a decrease in the percent of lymphocytes.

Both the glutamic-oxaloacetic transaminase (GOT) and the glutamic-pyruvic transaminase (GPT) of the plasma significantly increased 1 hr after AMOX administration. The values for both transaminases returned to about the levels of the control rats at 7 days.

The control rats as well as the four treated groups used for the experiment were from one shipment and had been housed in the animal quarters for more than 1 week. Their initial body weights were about the same and varied within a narrow range. AMOX was given to these rats at different times so that they could be all sacrificed on the same day. The rats that were given AMOX 7 days earlier lost appetite and lost weight.

5. Tissue Distribution and Excretion Pattern of Fluoride

Various tissues (including blood, liver, spleen, left kidney, heart, lung, brain, skeletal muscle, left thigh, both adrenals, thyroid, left femur, both upper and lower incisors), and excreta (including urine and feces) from control rats as well as from rats after AMOX administration were assayed for total fluoride. Each rat was anesthetized with ether. Blood was withdrawn as completely as possible from the abdominal aorta through a middle ventral incision and heparinized. Tissues were removed and kept frozen until ready for assay.

a. Tissue fluoride of control rats: Dietary intake of fluoride would no doubt affect the fluoride content in the various tissues. One single shipment of the rat laboratory chow, enough for the entire duration of absorption, distribution and excretion studies was ordered. A portion of the ordered feed in pellet form was ground and blended thoroughly with Model D Fitzpatric Comminuting Machine®. To investigate a possible difference between the pellet form laboratory chow and the well-blended chow on the effect of the distribution of total fluoride in various tissues, two groups of six male rats, weighing between 230 g and 290 g, were maintained on these two forms of feed for 2 weeks, respectively. Various tissues of these two groups of control rats were assayed for total fluoride content. There was no apparent difference in the total fluoride distribution in the various tissues. Therefore, the rat chow in pellet form was used for experiments of short duration (1 and 4 hr), whereas the blended chow was used for experiments of long duration in which rats were placed in individual metabolism cages for collection of urine and feces separately.

The concentrations of fluoride in the various tissues of the 12 control rats are presented in table 16. Average concentrations of 0.7 to 1.9 μg of total fluoride per g wet weight were found in the blood, liver, spleen, kidney, heart, lung, brain, muscle, adrenals, and thyroid. High concentrations of total fluoride were present in the femur and incisors, averaging 131 and 82 μg/g dry weight, respectively.

b. Tissue fluoride of rats after AMOX administration: Four groups of six male rats, weighing between 230 and 295 g, were given intraperitoneally 10 ml (35.6 mg) of AMOX per kg body weight. They were placed as groups of two in separate cages. Food and water were given ad libitum. One group of six rats were sacrificed at the end of 1 and 4 hr, and at 1 and 7 days after the administration of AMOX, respectively. Total fluoride concentrations in the various tissues of these rats were determined and are summarized in tables 17 through 20.

Tissue fluoride concentrations of the control rats and of rats after AMOX administration were compared. As shown in figure 5, the blood of control rats contained an average of 1.9 μg/ml of fluoride. The average fluoride concentration reached the peak of 17.6 μg/ml at 1 hr following AMOX administration. Thereafter, it decreased to 14.1 μg/ml at 4 hr and 6.7 μg/ml at 1 day. Seven days after AMOX administration, the average blood fluoride concentration decreased further to 2.7 μg/ml and was slightly higher than the level of the control rats. The fluoride concentrations of the liver, spleen, and kidney increased rapidly, as in the blood, and reached peak levels about 1 to 4 hr following AMOX administration. The concentrations of fluoride in these three tissues decreased to the ranges of control rats 7 days after AMOX administration.

Fluoride concentration was also determined in the plasma in addition to that in the whole blood. The amount of fluoride contained in the blood cells was calculated based on the hematocrit. As presented in tables 17 through 20, about one-half of the total blood fluoride was found in the blood cell fraction. AMOX administration did not affect the distribution of fluoride between the blood plasma and blood cells.

The average concentrations of fluoride in the heart, lung, brain, and muscle of rats increased during the first hour after AMOX administration as shown in figure 6. The concentrations of fluoride in the heart and lung continued to increase and reached peaks at 4 hr before returning to the control levels at 7 days. On the other hand, the fluoride concentrations of the brain and muscle remained at about the same levels at 4 hr as those at 1 hr after AMOX administration. At 7 days, muscle concentration of fluoride returned to the control levels, whereas concentration of the brain was about twice that of the control rats.

TABLE 16

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT)
OF CONTROL RATS

Tissue	Rat Weight, 230 - 290 g												Average
Blood[a]	2.1	2.5	1.5	1.5	1.8	1.7	2.2	2.5	1.5	1.8	1.8	2.2	1.9
Blood Cells[b,c]	50.0	48.7	49.1	46.4	45.6	63.0	51.7	45.7	-	-	-	-	50.0
Liver	1.1	1.3	1.0	0.9	1.5	0.9	1.1	1.8	0.9	0.9	1.5	1.6	1.2
Spleen	1.1	1.7	1.6	1.5	1.3	1.8	0.8	1.1	0.9	1.5	1.2	1.8	1.4
Kidney	0.9	1.1	0.7	0.6	0.7	1.1	0.9	1.0	0.7	0.5	0.6	1.1	0.8
Heart	0.8	1.8	1.3	1.2	0.8	1.1	0.7	1.8	1.2	1.3	0.8	1.2	1.1
Lung	1.6	1.4	1.1	0.8	1.0	0.8	0.8	1.5	1.0	0.7	1.1	0.8	1.1
Brain	1.1	1.2	0.8	0.4	-	0.6	1.0	1.2	0.9	0.4	0.7	-	0.8
Muscle	1.1	1.5	0.7	0.7	0.9	0.4	1.2	1.4	0.7	0.8	1.0	0.5	0.9
Adrenals[d]	0.5	-	0.6	-	0.9	-	1.0	-	-	-	-	-	0.7
Thyroid[d]	0.3	-	0.7	-	1.0	-	0.7	-	-	-	-	-	0.7
Femur[e]	145	130	174	126	144	80	154	135	134	119	130	103	131
Teeth[e]	98	83	84	72	140	61	56	40	66	121	-	-	82

a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood) - fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}} .$$

c/ Data were obtained from eight other control rats.

d/ Data were obtained from pooled sample of each two rats.

e/ μg/g dry weight.

TABLE 17

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS 1 HOUR AFTER INTRAPERITONEAL INJECTION OF AMOX

Tissue	Rat Weight (g) 250	235	265	285	240	Average
Blood[a]	16.4	18.2	21.8	15.8	15.6	17.6
Plasma[a]	14.8	15.2	15.2	17.6	13.6	15.3
Blood Cells[b]	47.7	58.0	68.6	42.1	57.2	54.7
Liver	12.0	25.1	19.3	18.1	10.9	16.7
Spleen	12.5	11.5	7.5	5.1	7.3	8.8
Kidney	7.5	7.7	8.1	11.4	6.1	8.2
Heart	6.8	2.8	3.8	4.2	4.0	4.3
Lung	4.2	2.9	6.9	1.3	1.7	3.4
Brain	3.6	2.4	2.4	2.8	3.2	2.9
Muscle	2.0	1.8	3.3	4.3	1.3	2.5
Adrenal[c]	-	-	-	-	-	1.0
Thyroid[c]	-	-	-	-	-	0.7

---

a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{(\text{fluoride in whole blood} - \text{fluoride in plasma} \times (1 - \text{hematocrit})}{\text{fluoride in whole blood}}.$$

c/ Data were obtained from pooled sample of all five rats.

TABLE 18

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS
4 HOURS AFTER INTRAPERITONEAL INJECTION OF AMOX

Tissue	Rat Weight (g) 280	245	280	275	250	230	Average
Blood[a]	15.6	14.0	13.2	12.0	15.2	14.8	14.1
Plasma[a]	15.6	13.6	12.4	11.2	14.8	9.6	12.9
Blood Cells[b]	47.1	48.6	46.5	46.8	52.3	67.0	51.3
Liver	12.3	12.9	9.2	13.1	8.5	11.1	11.2
Spleen	8.3	13.7	17.6	10.8	13.4	8.2	12.0
Kidney	6.5	9.2	7.5	9.0	8.1	8.3	8.1
Heart	8.7	6.3	8.9	6.0	8.6	10.5	8.2
Lungs	13.9	15.2	11.2	12.9	8.7	11.4	12.2
Brain	2.7	2.9	3.4	3.2	2.4	3.1	2.9
Muscle	2.3	2.8	2.7	2.9	2.5	2.6	2.6
Adrenals	2.8	2.0	2.0	3.7	1.7	-	2.4
Thyroid[c]	11.0	-	11.0	-	10.5	-	10.8
Femur[d]	316	209	354	291	345	406	321
Teeth[d]	428	110	139	81	-	63	162

a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood) - fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}}$$

c/ Data were obtained from pooled samples of each two rats.

d/ μg/g dry weight.

TABLE 19

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS
1 DAY AFTER INTRAPERITONEAL INJECTION OF AMOX

Tissue	Rat Weight (g) 275	230	230	230	255	275	Average
Blood[a]	6.8	6.8	7.6	8.0	4.0	7.2	6.7
Plasma[a]	7.2	5.0	8.0	6.8	4.0	4.8	6.0
Blood Cells[b]	35.4	53.6	32.6	48.2	41.0	56.7	44.5
Liver	1.1	1.7	1.3	1.4	2.2	2.1	1.7
Spleen	3.7	2.3	2.9	2.6	2.1	3.7	2.9
Kidney	4.1	6.8	5.6	4.7	2.6	6.4	5.0
Heart	3.2	4.9	3.5	5.2	3.1	3.8	3.9
Lung	1.6	2.2	1.8	2.2	1.7	1.7	1.9
Brain	2.5	1.8	3.7	1.1	-	2.9	2.4
Muscle	1.6	1.4	1.2	1.4	1.7	1.7	1.5
Adrenals	2.3	2.0	1.7	1.2	1.0	2.1	1.7
Thyroid	17.5	12.0	18.4	16.7	15.8	9.7	15.0
Femur[c]	464	454	489	551	634	286	479
Teeth[c]	381	573	606	649	545	680	572

---

a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood) - fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}}$$

c/ μg/g dry weight.

TABLE 20

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS
7 DAYS AFTER INTRAPERITONEAL INJECTION OF AMOX

Tissue	Rat Weight (g) 265	290	290	295	250	Average
Blood[a]	1.6	3.0	3.0	2.6	3.2	2.7
Plasma[a]	1.3	2.0	1.6	1.2	3.7	2.0
Blood Cells[b]	55.3	63.3	70.6	74.6	36.4	58.0
Liver	1.3	1.0	1.5	1.2	1.8	1.4
Spleen	1.0	0.8	1.3	0.7	0.9	0.9
Kidney	1.0	1.0	0.7	0.9	0.7	0.9
Heart	0.6	0.8	2.5	0.9	1.0	1.1
Lungs	1.0	1.1	0.7	0.7	1.3	1.0
Brain	1.6	1.6	1.7	1.6	1.4	1.6
Muscle	0.6	0.9	0.6	1.2	1.2	0.9
Adrenals	1.8	1.3	0.4	0.6	0.7	1.0
Thyroid	31.4	45.2	75.0	30.2	49.4	46.2
Femur[c]	556	439	265	637	525	485
Teeth[c]	382	288	444	220	408	348

[a] μg/ml.

[b] Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood) - fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}} .$$

[c] μg/g dry weight.

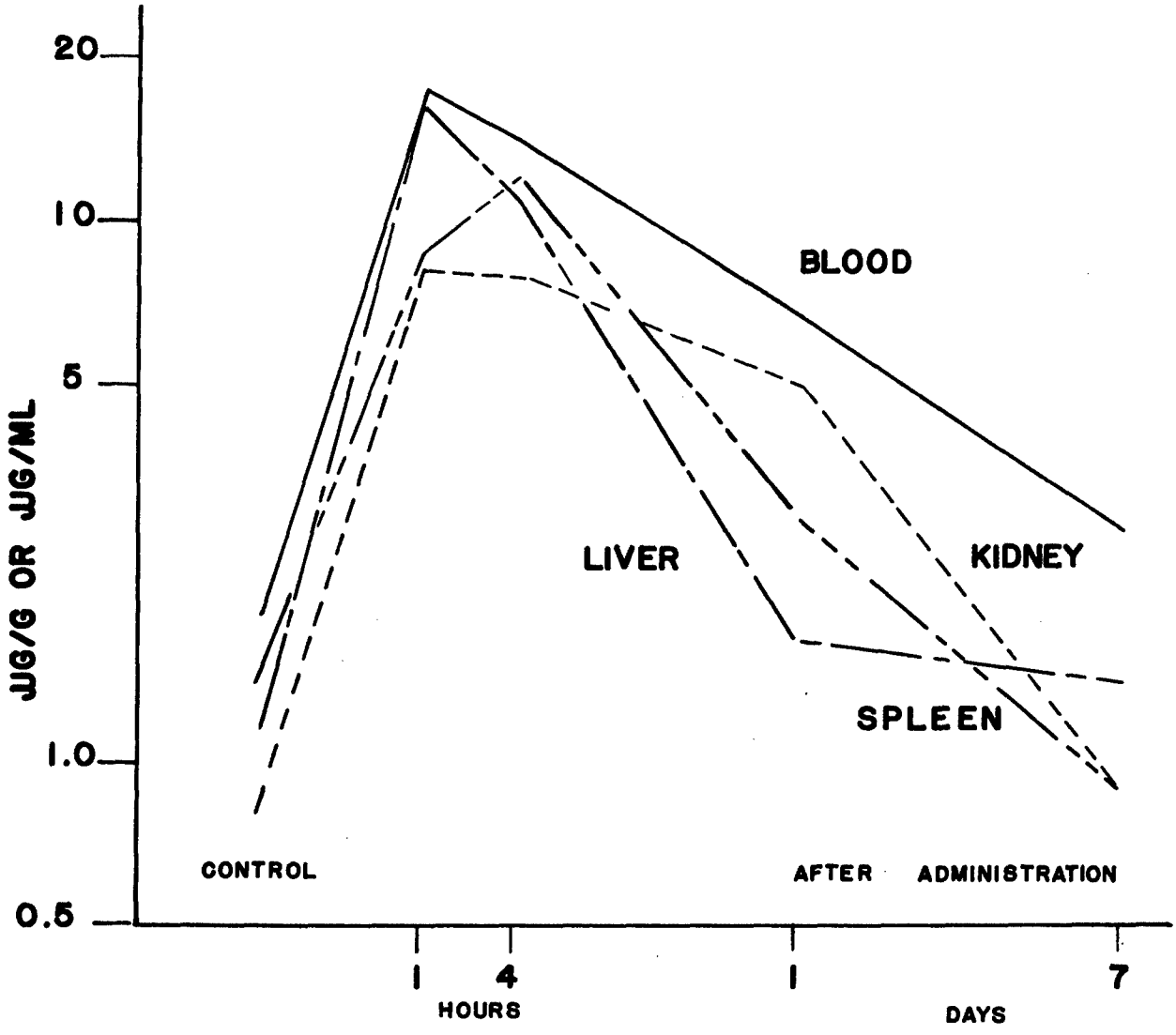

Figure 5 - Fluoride Concentration in Blood, Liver, Spleen and Kidney of Control Rats and Rats After AMOX Administration

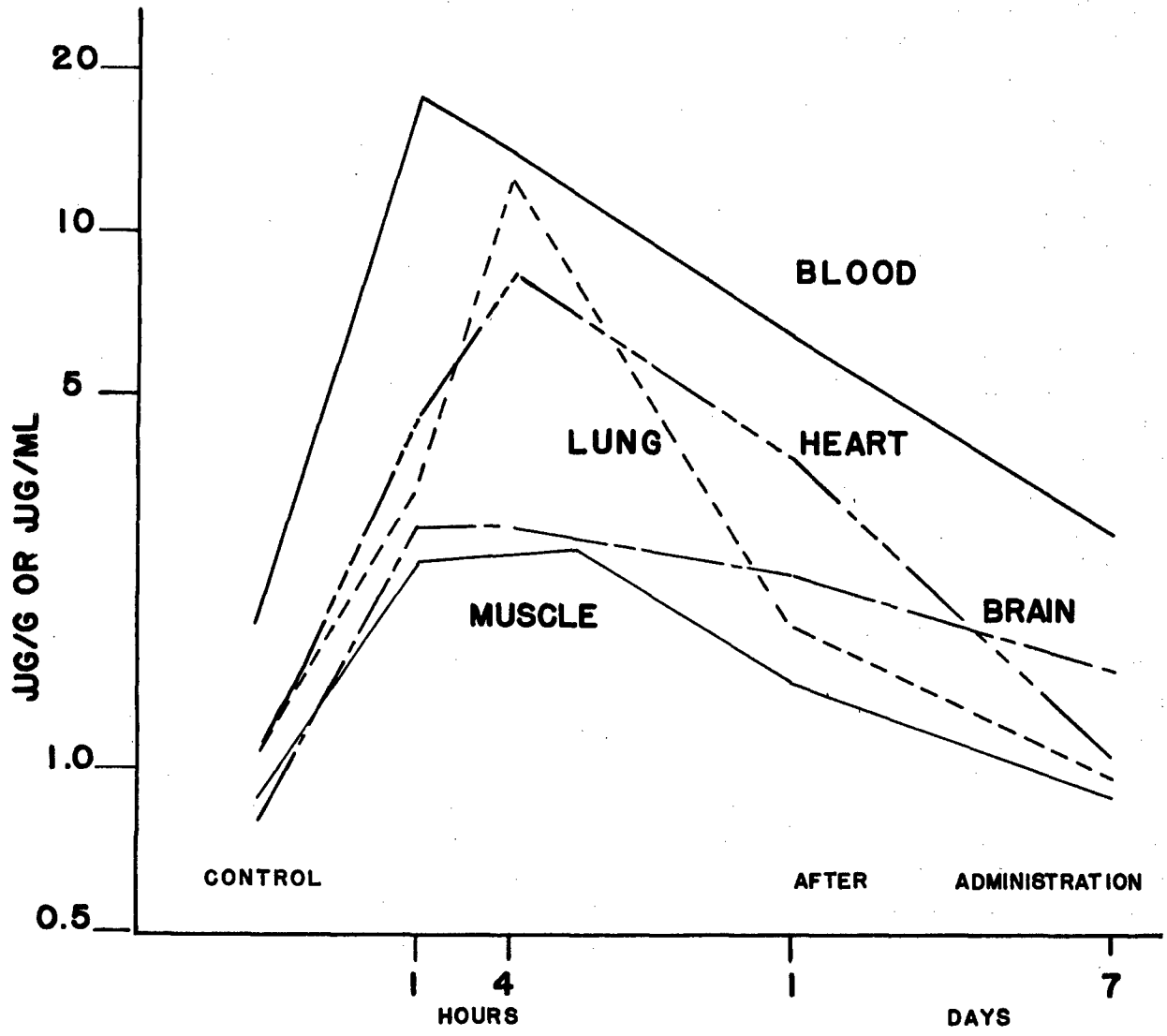

Figure 6 - Fluoride Concentration in Blood, Heart, Lung, Brain and Muscle of Control Rats and Rats After AMOX Administration

The average concentrations of fluoride in the adrenals, thyroid, femur, and teeth of control rats and rats after AMOX administration are shown in figure 7. The fluoride concentration of the adrenals changed only slightly after AMOX administration. On the other hand, the concentration of fluoride in the thyroid increased rapidly during the first 4 hr. It reached 10.8 μg/g wet weight at 4 hr and 15.0 μg/g at 1 day, a concentration approximately 2.2 times higher than the level of the blood of the same rats. The thyroid fluoride further increased and averaged 46.2 μg/g wet weight at 7 days. This concentration was 17 times higher than that of the blood. The administration of AMOX had no apparent effect on the thyroid weight of these rats. Iodine did not interfere with the analysis of fluoride.

Both the femur and the teeth of the control rats contained high concentrations of total fluoride, averaging 131 and 82 μg/g dry weight, respectively. After AMOX administration, the average fluoride concentration of the femur increased to 321 and 479 μg/g dry weight at 4 hr and 1 day, respectively. At 7 days, the fluoride concentration remained high at 485 μg/g. On the other hand, the average fluoride concentration of the teeth increased to 162 and 572 μg/g dry weight at 4 and 24 hr, respectively, and decreased to 348 μg/g at 7 days.

As the concentrations of fluoride in the thyroid, femur and teeth remained at significantly higher levels 7 days after AMOX administration than those of the control rats, the disappearance of fluoride from these tissues was further investigated. A group of six male rats, weighing between 230 and 275 g, was administered AMOX intraperitoneally with 10 ml(35.6 mg)/kg body weight. At the end of 2, 4, and 6 weeks, two rats were sacrificed at each interval. The thyroid, femur and teeth were removed and assayed for fluoride concentration. As shown in figure 7, the concentrations of fluoride in the femur and teeth decreased to the normal levels of control rats in about 2 weeks whereas that in the thyroid did not return to the normal ranges until 4 to 6 weeks.

c. Urinary and fecal excretion of fluoride: A group of seven male rats, weighing between 180 and 220 g, were injected intraperitoneally with 10 ml (35.6 mg) of AMOX per kg body weight. Each rat was placed in an individual metabolism cage. Food and water were available at all times. The daily excretions of urine and feces were collected separately for 7 days and kept in the refrigerator until time of assay.

The excretions of fluoride in the urine and feces of rats after AMOX administration are summarized in table 21. After a single intraperitoneal injection of 35.6 mg of AMOX per kg body weight, an average of 61.1%, ranging from 53.1 to 69.4%, of the administered fluoride was excreted in the urine in 7 days. The majority of the urinary excretion occurred during the first 2 days after AMOX administration, averaging 33.6 and 20.2% of the dose, respectively.

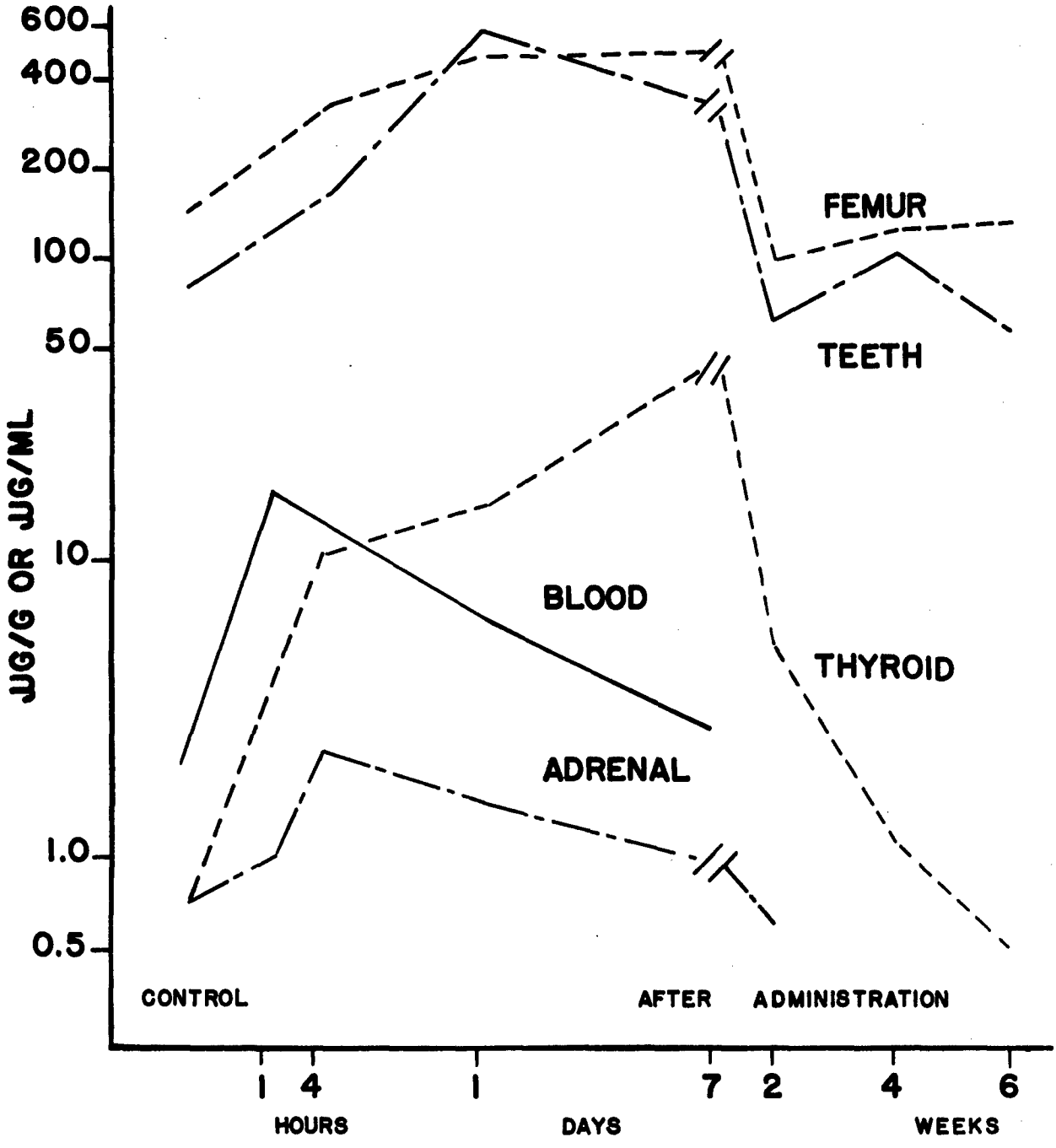

Figure 7 - Fluoride Concentrations in Blood, Adrenals, Thyroid, Femur and Teeth of Control Rats and Rats After AMOX Administration

TABLE 21

THE AMOUNT OF FLUORIDE EXCRETED IN URINE AND FECES (PERCENT OF DOSE) AND URINE VOLUME OF RATS AFTER INTRAPERITONEAL INJECTION OF AMOX

Weight of Rat (g)	Urinary Excretion Days After AMOX Administration 1	2	3	4	5	6	7	Total	Fecal Excretion 7 Days
190	30.8	17.8	1.3	2.1	2.0	1.9	0.9	56.8	16.9
190	39.1	14.2	2.4	2.0	0.9	1.2	1.2	61.0	5.7
200	30.3	25.1	1.2	1.5	1.5	1.4	1.4	62.4	14.0
180	32.5	28.3	0.9	1.7	1.1	1.0	0.6	66.1	5.9
180	39.7	21.4	2.4	1.7	2.2	1.2	0.8	69.4	9.1
200	32.6	18.8	1.5	1.1	2.3	1.9	0.8	59.0	6.9
220	29.9	16.0	1.6	1.8	1.9	1.4	0.5	53.1	9.7
Average	33.6	20.2	1.6	1.7	1.7	1.4	0.9	61.1	9.7

Urine Volume, ml. Days After AMOX Administration 1	2	3	4	5	6	7	Total
10.0	14.0	10.0	11.0	20.0	15.0	10.0	90.0
20.0	14.0	11.0	19.0	21.0	19.0	11.0	115.0
12.0	16.0	14.0	16.0	24.0	8.0	7.0	97.0
10.5	17.0	13.0	18.0	24.0	20.0	12.0	114.5
9.5	16.0	9.0	11.0	13.0	14.0	10.0	82.5
11.5	11.0	13.0	14.0	13.0	9.0	13.0	84.5
17.0	16.0	20.0	18.0	25.0	20.0	20.0	136.0
Average 12.9	14.9	12.9	15.3	20.0	15.0	11.8	102.8

These same rats excreted an average of 9.7% of the administered fluoride in the feces in 7 days. Six control rats excreted small amounts of fluoride in the urine and feces, averaging 4.2 and 1.8 μg per day, respectively.

The urine volumes from these rats after AMOX administration are also presented in table 21. These rats excreted an average of 102.8 ml of urine, ranging from 82.5 ml to 136.0 ml, during the 7-day collecting period. The average daily urine volume varied from 11.8 ml to 20.0 ml. Before AMOX administration, these rats excreted an average of 10.6 ml to 13.2 ml of urine daily during a period of 5 days.

B. TAMA

1. Acute Intraperitoneal and Oral Toxicities

Freon 113 was employed as a vehicle for study of the intraperitoneal and oral toxicities of TAMA.

a. Toxicity of Freon 113: Both the intraperitoneal and oral toxicities of Freon 113 were studied in groups of five rats and five mice. No rats died after as much as 10 ml of Freon 113 per kg body weight was given by either route. However, mice were extremely sensitive to Freon 113. A dose of 2.5 ml/kg body weight killed all the mice when given intraperitoneally, and two out of five mice died when the same dose was given orally. On the basis of this result, the toxicities of TAMA were investigated only in rats. All doses were given in Freon 113 at a volume of 2 ml/kg body weight.

b. Median lethal doses: A total of more than 300 rats, weighing between 165 and 245 g, were given various doses of TAMA intraperitoneally or orally. All doses were administered in Freon 113 at a volume of 2 ml/kg body weight, and all animals were observed for 7 days. The dose levels and LD_{50} of TAMA after intraperitoneal administration are summarized in table 22. The LD_{50} for female rats at the end of 7 days after intraperitoneal injection was 94 mg/kg body weight, whereas that of the male rats was significantly higher; i.e., 140 mg/kg body weight. The TAMA was less toxic to rats after oral administration. Only 2 of 16 female rats that were given the highest dose of TAMA (200 mg/kg body weight) died in 7 days, whereas 1 of 12 males died after the same dose.

c. Symptoms: Within minutes after intraperitoneal administration of TAMA, all rats showed abdominal tenderness, great distress, increased excitability, and reacted quickly to sound and physical stimuli. They fought each other and attacked any unusual objects in their vicinity. When the cover was removed from the cage, they attempted to jump out and escape. After a few hours, the rats started to show weakness, depression and loss of appetite.

TABLE 22

DOSE LEVELS AND LD_{50} OF TAMA FOR RATS AFTER INTRAPERITONEAL OR ORAL ADMINISTRATION

	mg of TAMA/kg Body Weight						
	50	75	100	125	150	175	LD_{50} (95% CL) mg/kg
Intraperitoneal Administration							
Females, 165 - 245 g	0/7[a]	5/16	9/19	13/16	14/16		94 (84-105)
Males, 190 - 245 g	-	0/12	3/12	6/12	7/12	9/16	140 (122-168)
	100	125	150	175	200		
Oral Administration							
Females, 180 - 225 g	0/4	0/20	0/20	0/20	2/16		
Males, 190 - 245 g	0/4	0/18	0/18	0/18	1/12		

a/ Number of deaths/number of treated.

They died slowly in various distress positions. The rats that survived the observation period of 7 days usually lost weight and remained weak. After oral administration of higher doses of TAMA, the rats showed some degree of abdominal distress.

d. Effect on adrenal weight: The adrenals of rats after intraperitoneal administration of 50 mg/kg of TAMA as a 2.5% solution in Freon 113 and of rats after Freon 113 alone were removed and weighed. The results are presented in table 23. The adrenal weight of control rats averaged 187 $\pm$ 5 mg/kg body weight (table 14). The average adrenal weights of rats after administration of TAMA in Freon 113 or Freon 113 alone were smaller at 1 hr, increased at 4 and 24 hr, and dropped at 7 days. However, the change in weight was much greater at 1 day after TAMA administration. At this time, the adrenal weight after administration of TAMA in Freon 113 was significantly higher that that following administration of Freon 113 alone.

3. Effect on Blood Pressure

The effect of TAMA on blood pressure and EKG was likewise investigated in rats in the same manner as that described for AMOX. One male rat weighing 300 g was given intraperitoneally 150 mg/kg of TAMA as a 7.5% solution in Freon 113. Freon 113 alone was given to another male rat weighing 280 g. No appreciable change in blood pressure of either rat was observed except the immediate drop after administration due to mechanical handling. A second dose of TAMA in Freon 113 and Freon 113 alone was administered to the same rat after 2 hr. The blood pressure of each rat was down about 50 mm Hg when they were sacrificed 2 hr after the second dose.

4. Clinical Laboratory Studies on TAMA

Four groups of male rats, weighing between 200 and 250 g, were given intraperitoneally 50 mg of TAMA per kg body weight (2 ml of a 2.5% solution of TAMA in Freon 113 per kg). They were placed in separate cages in groups of two. At the end of 1, and 4 hr and 1 and 7 days, blood from each of six rats was taken for various clinical laboratory studies as described for AMOX. Four additional groups of rats were also given intraperitoneally 2 ml of Freon 113 per kg body weight. They were sacrificed accordingly and their blood served as controls. The results are summarized in tables 24 and 25, respectively.

At time of termination, the abdominal cavity of all treated rats contained various amounts of clear exudates as in the rats after AMOX administration. There was no change in any clinical laboratory tests which could be attributed to the effect of TAMA alone.

TABLE 23

WEIGHTS OF ADRENALS (mg/kg BODY WEIGHT) OF MALE RATS AFTER INTRAPERITONEAL INJECTION OF TAMA IN FREON 113 OR IN FREON 113 ALONE

	Time After Administration			
	1 Hr	4 Hr	1 Day	7 Days
TAMA (50 mg/kg) in 2 ml/kg of Freon 113				
	152	193	279	200
	196	174	258	174
	216	212	285	225
	194	206	263	226
	155	198	290	233
	153	210	243	195
Average ± S E	178 ± 11	199 ± 6	270 ± 7	209 ± 9
			p* < 0.01	p* < 0.1
2 ml/kg of Freon 113				
	115	201	165	152
	135	208	173	196
	163	205	213	216
	184	217	216	194
	165	178	173	155
	127	179	284	153
Average ± S E	155 ± 8	198 ± 6	204 ± 18	178 ± 11

* Student "t" test (ref 17): The average adrenal weights after administration of TAMA in Freon 113 were compared with those of the respective groups following administration of Freon 113 above.

TABLE 24

CLINICAL LABORATORY DATA OF RATS AFTER INTRAPERITONEAL INJECTION OF TAMA IN FREON 113

	Control	Time After TAMA Administration			
		1 Hr	4 Hr	1 Day	7 Days
No. of Animals	8	6	6	6	6
Body Weight (g)	185 - 220	200 - 250	190 - 220	185 - 190	200 - 245
Bleeding Time (sec)	199 ± 21	155 ± 21	175 ± 23	200 ± 25	215 ± 14
Clotting Time (sec)	116 ± 22	86 ± 27	93 ± 14	71 ± 13	103 ± 15
RBC (x 10^6)	5.85 ± 0.13	8.58 ± 0.38	6.34 ± 0.13	7.21 ± 0.18	8.18 ± 0.37
WBC (x 10^3)	7.9 ± 0.5	4.7 ± 0.9	6.9 ± 0.9	7.4 ± 1.3	6.9 ± 0.7
Lymphocytes (%)	82 ± 4	82 ± 4	56 ± 6	67 ± 7	77 ± 3
Neutrophils (%)	16 ± 4	16 ± 3	43 ± 6	29 ± 6	20 ± 2
Eosinophils (%)	0	0	0	0	1
Basophils (%)	0	0	0	0	0
Monocytes (%)	2	2	1	4	2
Hematocrit (vol %)	43 ± 1	52 ± 3	46 ± 1	48 ± 1	48 ± 2
Hemoglobin (g%)	13.3 ± 0.2	16.9 ± 0.8	14.7 ± 0.3	14.5 ± 0.3	15.6 ± 0.5
MetHb (g%)	0	0	0	0	0
MCV (μ^3)	73.8 ± 1.5	60.9 ± 1.1	72.2 ± 0.6	67.2 ± 1.3	59.1 ± 0.9
MCHb ($\mu\mu$g)	22.5 ± 0.4	19.7 ± 0.4	23.3 ± 0.4	20.3 ± 0.4	19.2 ± 0.3
MCHbC (%)	30.8 ± 0.2	32.3 ± 0.4	31.0 ± 0.6	33.3 ± 0.5	32.5 ± 0.3
GOT (S F Units)	99 ± 14	208 ± 21	296 ± 37	195 ± 35	96 ± 8
GPT (S F Units)	38 ± 2	48 ± 4	99 ± 21	89 ± 23	36 ± 1

TABLE 25

CLINICAL LABORATORY DATA OF RATS AFTER INTRAPERITONEAL INJECTION OF FREON 113

		Time After Freon Administration			
	Control	1 Hr	4 Hr	1 Day	7 Days
No. of Animals	8	6	6	6	6
Body Weight (g)	185 - 220	270 - 300	180 - 220	250 - 300	195 - 220
Bleeding Time (sec)	199 ± 21	240 ± 35	140 ± 18	250 ± 5	215 ± 28
Clotting Time (sec)	116 ± 22	140 ± 12	83 ± 23	106 ± 19	98 ± 17
RBC (x 10^6)	5.85 ± 0.13	7.50 ± 0.33	6.33 ± 0.1	6.37 ± 0.06	8.46 ± 0.38
WBC (x 10^3)	7.9 ± 0.5	5.4 ± 0.5	6.2 ± 1.0	9.8 ± 1.1	5.4 ± 0.7
Lymphocytes (%)	82 ± 4	77 ± 2	52 ± 5	80 ± 5	78 ± 5
Neutrophils (%)	16 ± 4	22 ± 2	46 ± 4	20 ± 5	18 ± 3
Eosinophils (%)	0	0	0	0	2
Basophils (%)	0	0	0	0	0
Monocytes (%)	2	1	2	0	2
Hematocrit (vol %)	43 ± 1	48 ± 2.0	46 ± 1	43 ± 0.6	49 ± 1
Hemoglobin (g %)	13.3 ± 0.2	15.4 ± 0.6	14.6 ± 0.3	13.6 ± 0.2	15.7 ± 0.4
MetHb (g %)	0	0	0	0	0
MCV (μ^3)	73.8 ± 1.5	64.7 ± 1.0	72.8 ± 1.3	67.5 ± 0.8	58.1 ± 1.6
MCHb ($\mu\mu$g)	22.5 ± 0.4	20.6 ± 0.4	23.0 ± 0.4	21.3 ± 0.3	18.4 ± 0.6
MCHbC (%)	30.8 ± 0.2	31.1 ± 0.4	31.6 ± 1.1	31.4 ± 0.4	32.0 ± 0.4
GOT (S F Units)	99 ± 14	258 ± 24	330 ± 32	383 ± 21	106 ± 4
GPT (S F Units)	38 ± 2	63 ± 12	33 ± 7	91 ± 18	29 ± 2

5. Effect on Mucous Membranes

The Draize technique, based upon application of the test substance to rabbit eyes (ref 18), was employed to study the possible irritating effect of TAMA. One-tenth ml of a 5 or 10% TAMA solution in Freon 113 was instilled in the left eye of two different rabbits. These two dose levels gave 5 and 10 mg of TAMA to each eye, respectively. The right eye of both rabbits was given 0.1 ml of Freon 113 likewise and served as controls. Observations of injuries were made with the unaided eye on the cornea, the iris, and the bulbar and palpebral conjunctivae at 1 and 4 hr, and at 1, 2, 3, 4, and 7 days after treatment. The TAMA treated eye of both rabbits showed no obvious ocular reactions except mild redness of the conjunctivae which was also seen in the control eye of both rabbits.

6. Tissue Distribution and Excretion Pattern of Fluoride

Various tissues and excreta from rats after Freon 113 administration and from rats after administration of TAMA in Freon 113 were assayed for total fluoride as described for rats after AMOX administration.

a. Tissue fluoride of rats after Freon 113 administration: Various tissues of all rats that were used for clinical laboratory studies were removed and kept in the refrigerator. These rats were given Freon 113 intraperitoneally at a dose of 2 ml/kg of body weight. One group of six rats was sacrificed at the end of 1 and 4 hr, and 1 and 7 days after administration. Total fluoride concentrations from two rats of each group were determined and are presented in table 26. The fluoride concentrations in the various tissues of untreated control rats are recorded in table 16. A dose of 950 mg of fluoride per kg body weight contained in the Freon 113 was administered to these rats. Only a very small amount of the administered fluoride was absorbed by these rats. The fluoride concentration of each tissue varied only slightly when these rats were sacrificed at various times following the administration of Freon 113. The composite averages of the tissue fluoride from these eight rats were used as the reference baseline levels for those of rats after administration of TAMA in Freon 113.

b. Tissue fluoride of rats after administration of TAMA in Freon 113: Total fluoride concentrations were determined in the various tissues of all rats that were used for clinical laboratory studies. TAMA was administered to these rats at 50 mg/kg body weight in a volume of 2 ml/kg of Freon 113. At the end of 1 and 4 hr, and 1 and 7 days after administration, various tissues were removed from each rat and kept in the refrigerator until assay for total fluoride. The results are presented in tables 27 through 30.

TABLE 26

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS AFTER INTRAPERITONEAL INJECTION OF FREON 113

Rat Weight, 190 - 300 g

	Time After Injection								
	1 Hr		4 Hr		1 Day		7 Days		Average
Blood[a]	3.7	3.5	3.7	3.3	3.1	2.8	1.2	0.9	2.8
Plasma[a]	3.1	3.0	3.4	3.0	2.7	2.7	1.2	0.8	2.5
Blood Cells[b]	62.3	59.7	55.9	50.0	53.0	47.9	46.0	57.3	54.0
Liver	1.9	2.0	1.7	1.7	1.1	0.8	0.8	0.5	1.3
Spleen	1.6	1.1	1.2	0.7	1.2	0.7	0.7	0.6	1.0
Kidney	2.3	1.8	0.7	2.4	1.2	1.5	1.6	0.8	1.5
Heart	2.0	2.2	2.4	0.8	0.9	0.9	2.2	0.5	1.5
Lung	0.9	1.2	0.8	0.7	1.2	0.6	1.1	0.6	0.9
Brain	0.9	0.6	0.9	0.6	0.7	0.7	0.6	0.6	0.7
Muscle	1.6	2.1	2.1	2.0	1.6	1.5	1.0	0.8	1.5
Adrenals	0.5	-	0.6	0.8	0.8	0.7	0.6	0.7	0.7
Thyroid	-	0.7	2.6	1.2	1.1	1.5	0.8	1.0	1.3
Teeth[c]	136	105	134	121	130	111	124	114	122
Femur[c]	77	114	63	93	99	93	79	71	86

a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood) - fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}} .$$

c/ μg/g dry weight.

TABLE 27

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS
1 HOUR AFTER INTRAPERITONEAL INJECTION OF
TAMA IN FREON 113

Tissue	Rat Weight (g)						Average
	250	245	200	230	230	220	
Blood[a]	25.2	19.2	16.0	27.2	20.0	29.6	22.9
Plasma[a]	25.2	22.4	12.4	25.2	-	26.4	22.3
Blood Cells[b]	55.0	39.4	69.4	51.8	-	63.4	55.8
Liver	30.9	33.1	17.7	17.0	13.5	22.5	22.5
Spleen	18.3	24.6	37.4	30.5	14.5	13.7	23.2
Kidney	10.6	10.2	5.7	12.7	13.8	12.9	10.9
Heart	5.6	6.1	7.9	3.7	5.1	7.8	6.0
Lung	2.2	1.9	2.0	1.5	3.1	4.6	2.6
Brain	2.2	1.2	1.3	1.8	0.9	1.9	1.6
Muscle	3.0	2.4	1.2	1.1	1.2	1.6	1.8
Adrenals	0.7	1.0	0.5	0.9	3.0	1.5	1.3
Thyroid	3.0	1.1	3.3	0.9	1.1	1.0	1.7
Femur[c]	368	439	-	348	407	441	401
Teeth[c]	212	230	165	295	123	129	192

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a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood) - fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}}.$$

c/ μg/g dry weight.

TABLE 28

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS
4 HOURS AFTER INTRAPERITONEAL INJECTION OF
TAMA IN FREON 113

Tissue	Rat Weight (g) 200	220	190	205	200	200	Average
Blood[a]	31.6	48.0	39.2	25.2	26.2	27.2	32.9
Plasma[a]	27.2	47.6	26.4	24.0	26.2	25.0	29.4
Blood Cells[b]	55.3	46.5	65.0	44.8	44.0	51.5	51.2
Liver	32.1	32.0	26.9	37.6	18.7	14.8	27.0
Spleen	30.6	12.2	43.7	17.3	10.5	27.5	23.6
Kidney	11.0	7.4	12.8	9.8	10.8	8.8	10.1
Heart	16.6	11.4	11.0	8.7	9.5	6.6	10.6
Lung	7.7	5.1	7.1	5.9	11.2	8.6	7.6
Brain	1.5	1.9	1.8	2.0	3.8	4.6	2.6
Muscle	1.9	2.9	0.9	1.2	2.7	1.5	1.9
Adrenals	1.8	1.2	0.8	1.1	2.4	1.2	1.4
Thyroid	10.8	40.6	6.4	20.9	14.6	18.3	18.6
Femur[c]	293	601	403	797	507	509	518
Teeth[c]	89	239	544	469	319	530	365

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a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood)- fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}} .$$

c/ μg/g dry weight.

TABLE 29

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS
1 DAY AFTER INTRAPERITONEAL INJECTION OF
TAMA IN FREON 113

Tissue	Rat Weight (g)						Average
	190	190	165	175	165	180	
Blood[a]	14.2	13.2	14.8	9.6	11.2	15.8	13.1
Plasma[a]	13.6	13.2	14.0	9.2	10.0	10.0	11.7
Blood Cells[b]	49.3	46.1	56.5	52.1	62.5	65.2	55.1
Liver	3.9	5.1	2.0	6.2	3.4	7.2	4.6
Spleen	8.7	6.4	9.8	7.5	10.4	6.9	8.3
Kidney	12.0	7.5	6.1	7.1	5.4	6.9	6.9
Heart	5.4	4.9	4.3	4.3	5.3	3.3	4.7
Lung	4.0	1.7	2.5	5.1	1.0	1.6	2.7
Brain	1.6	1.7	1.5	1.4	1.4	1.9	1.6
Muscle	1.6	1.0	1.6	1.4	1.2	1.1	1.3
Adrenals	-	1.5	0.7	0.5	1.8	0.6	1.0
Thyroid	43.6	29.8	51.6	37.2	43.1	32.4	39.6
Femur[c]	1045	406	603	798	798	994	774
Teeth[c]	769	498	691	753	459	612	630

a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood) - fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}}$$

c/ μg/g dry weight.

TABLE 30

TISSUE FLUORIDE CONCENTRATION (μg/g WET WEIGHT) OF RATS
7 DAYS AFTER INTRAPERITONEAL INJECTION
OF TAMA IN FREON 113

Tissue	Rat Weight (g) 220	270	230	225	240	200	Average
Blood[a]	1.6	1.9	1.8	1.3	1.4	2.0	1.7
Plasma[a]	1.2	1.8	1.7	0.8	0.9	2.1	1.4
Blood Cells[b]	61.9	56.8	45.6	68.8	64.7	41.5	56.8
Liver	1.2	1.3	-	1.4	1.7	1.8	1.5
Spleen	0.9	0.9	0.7	0.6	0.5	0.8	0.7
Kidney	0.7	0.7	0.4	1.1	0.9	1.1	0.8
Heart	1.0	0.7	0.6	1.0	1.7	1.1	1.0
Lung	0.9	0.7	0.6	0.9	0.7	1.4	0.9
Brain	0.6	1.0	1.3	1.3	0.5	1.0	0.9
Muscle	0.6	0.9	0.8	0.9	0.4	0.5	0.7
Adrenals	0.6	-	0.6	0.8	0.9	0.8	0.7
Thyroid	26.8	45.1	47.0	15.8	30.5	44.3	28.2
Femur[c]	536	437	429	606	602	699	552
Teeth[c]	123	204	271	92	147	338	196

a/ μg/ml.

b/ Percent of total blood fluoride

$$= \frac{\text{(fluoride in whole blood) - fluoride in plasma x (1 - hematocrit)}}{\text{fluoride in whole blood}} .$$

c/ μg/g dry weight.

Tissue fluoride concentrations in the Freon 113 administered control rats were compared with those in rats after administration of TAMA in Freon 113. Fluoride concentrations of the blood, liver, spleen, and kidney are shown in figure 8. The average concentration of fluoride in the blood of Freon 113 administered rats was 2.8 μg/ml. The fluoride concentration increased to 22.9 μg/ml at 1 hr after administration of TAMA in Freon 113 and reached peak level of 32.9 μg/ml at 4 hr. Thereafter, the fluoride concentration of the blood decreased to 13.1 μg/ml at 1 day and 1.7 μg/ml at 7 days. Concentrations of fluoride in the liver, spleen, and kidney increased quickly after administration of TAMA in Freon 113 and reached peak levels about 1 to 4 hr. Fluoride concentrations of these three tissues returned to the normal levels of control rats 7 days after administration.

As shown in tables 27 through 30, distribution of fluoride in blood was not altered after administration of TAMA. Blood cells contained about one-half of the total blood fluoride.

Figure 9 shows that the average fluoride concentrations of the heart and lung increased during the first 4 hr after administration of TAMA in Freon 113 and returned to control levels at 7 days. On the other hand, the fluoride concentrations in the brain and muscle increased only slightly during the first 4 hr and dropped thereafter.

Fluoride concentration of the thyroid, femur, and teeth increased more rapidly than that in the other tissues after administration of TAMA in Freon 113. The results are shown in figure 10. The fluoride concentration of the thyroid increased rapidly during the first 4 hr and reached a peak level of 39.6 μg/g wet weight at 1 day. At this time, the fluoride concentration was about three times that of the blood. The concentration of fluoride in the thyroid was 28.2 μg/g wet weight at 7 days, a concentration which was 16 times higher than that of the blood of the same rats. The thyroid weight of these rats was not apparently altered after TAMA administration. The fluoride concentration of the adrenals increased only slightly after administration of TAMA.

The concentrations of fluoride in the femur and teeth of rats after administration of Freon 113 alone averaged 122 μg and 86 μg/g dry weight, respectively. After administration of TAMA in Freon 113, the femur contained 401, 518, 774 and 552 μg of total fluoride per g dry weight at 1 and 4 hr, and at 1 and 7 days, respectively. On the other hand, the average fluoride concentration in the teeth of these rats was 192, 365, 630 and 196 μg/g dry weight at the same time intervals after administration of TAMA in Freon 113, respectively.

The disappearance of fluoride from the thyroid, femur and teeth was further investigated in a group of six male rats weighing between 180 and 210 g.

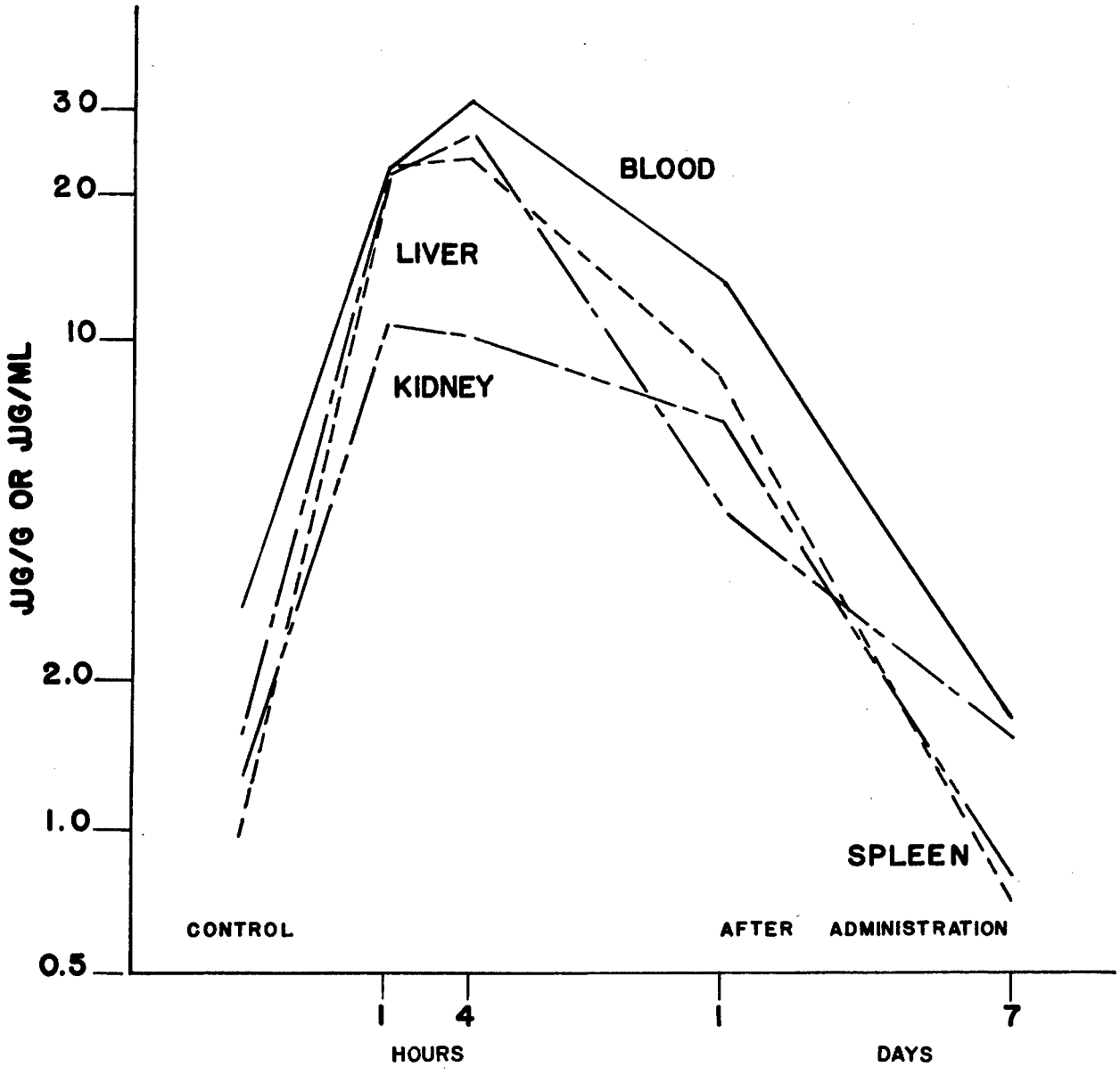

Figure 8 - Fluoride Concentrations in Blood, Liver, Spleen and Kidney of Contro. Rats and Rats After TAMA Administration

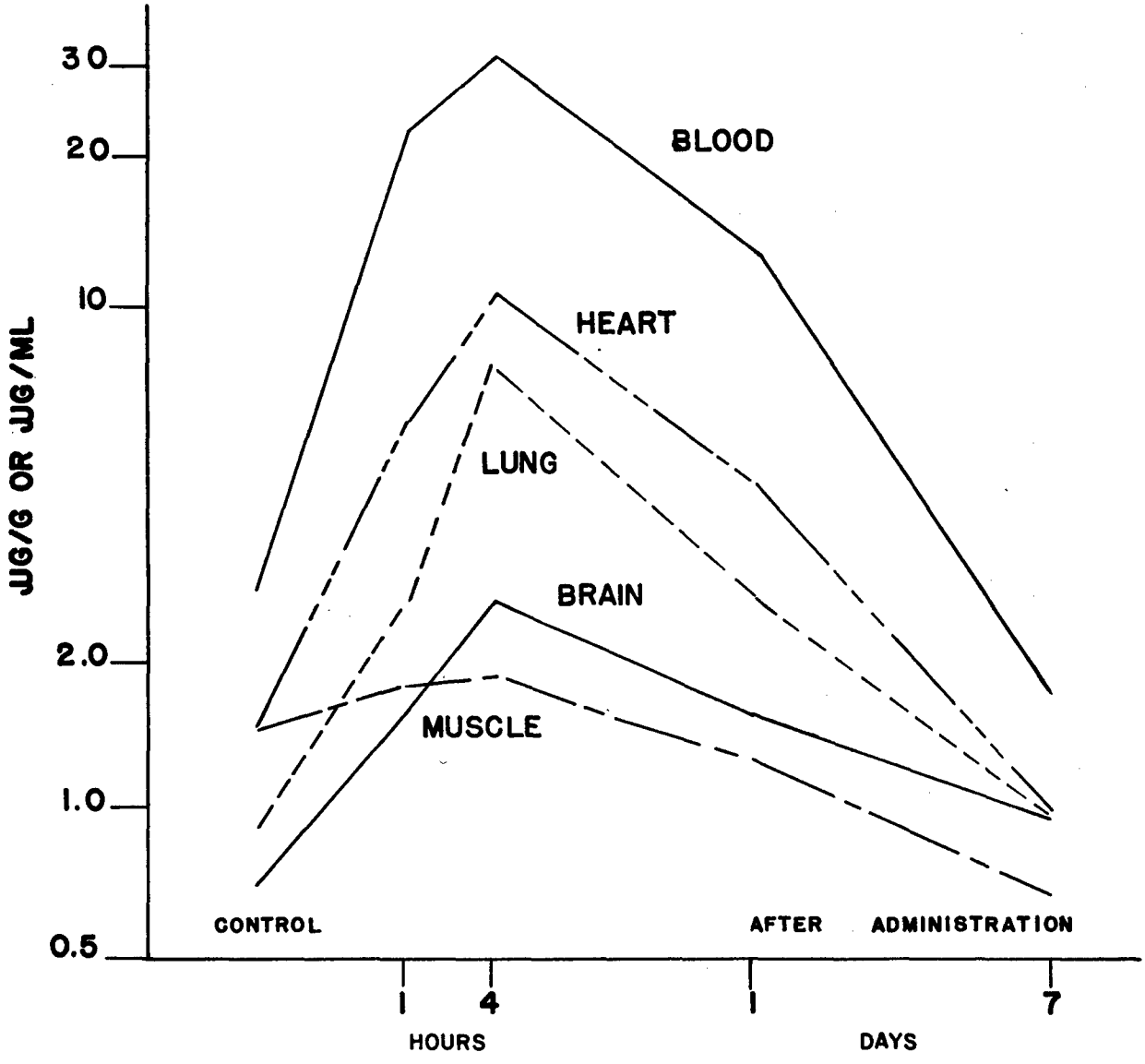

Figure 9 - Fluoride Concentration in Blood, Heart, Lung, Brain and Muscle of Control Rats and Rats After TAMA Administration

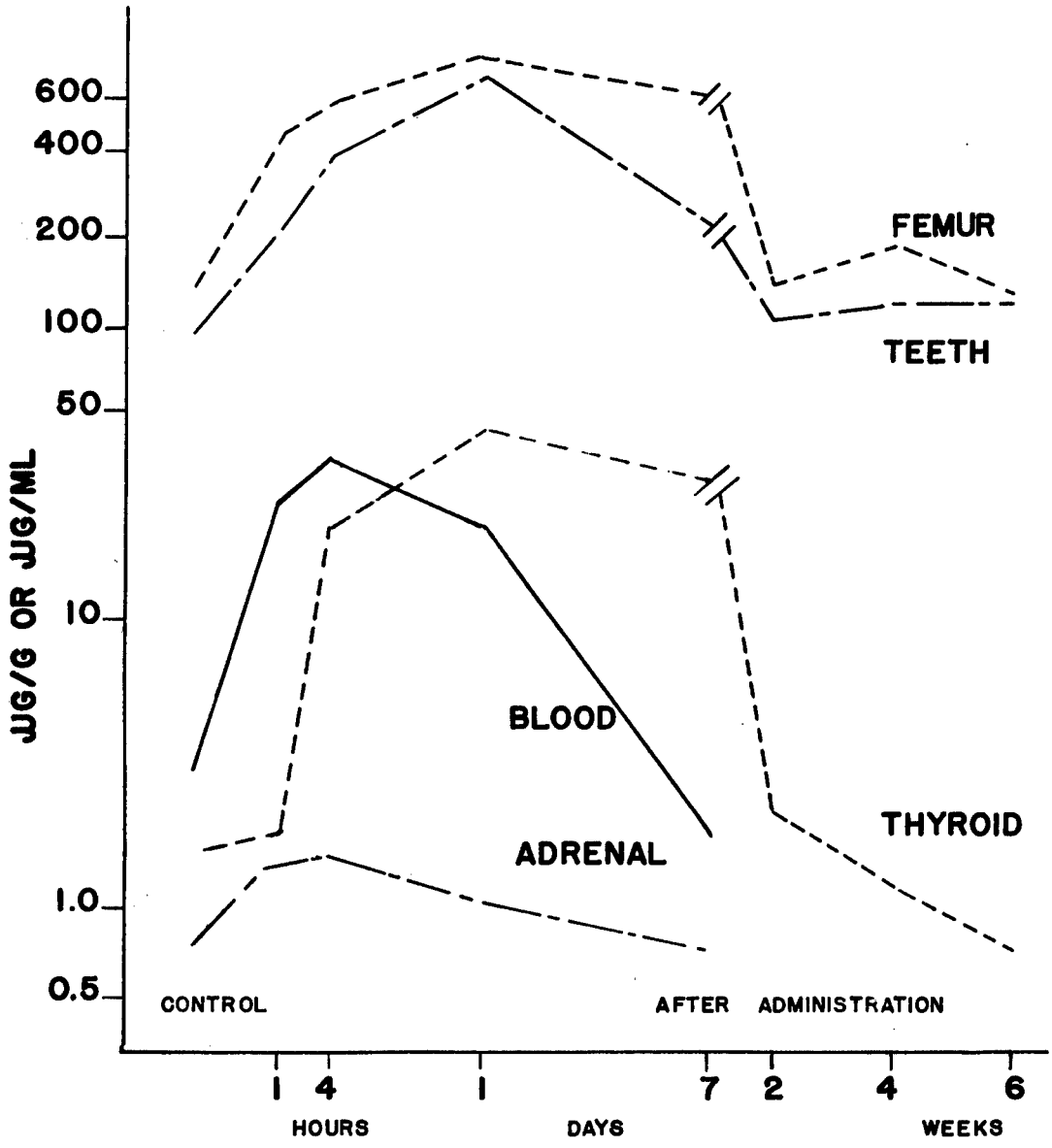

Figure 10 - Fluoride Concentration in Blood, Adrenals, Thyroid, Femur and Teeth of Control Rats and Rats After TAMA Administration

A dose of 50 mg of TAMA per kg body weight in a volume of 2 ml/kg of Freon 113 was administered intraperitoneally to each rat. They were placed in separate cages in groups of two. Two rats each were sacrificed at the end of 2, 4, and 6 weeks. Total fluoride concentrations of the thyroid, femur and teeth were determined and are shown in figure 10. Concentrations of fluoride in the femur and teeth returned to the levels of control rats after about 2 weeks, whereas that in the thyroid did not reach the range of control rats until 6 weeks.

c. Urinary and fecal excretion of fluoride: A group of six male rats, weighing between 180 g and 210 g, was administered intraperitoneally with 50 mg of TAMA per kg body weight in a volume of 2 ml/kg of Freon 113. Each rat was kept in a separate metabolism cage. Feed and water were provided ad libitum. Excretions of urine and feces were collected separately for 7 days. They were kept in the refrigerator for assay.

Total fluoride excretions in the urine and feces of these rats were determined and are summarized in table 31. After a single intraperitoneal injection of 50 mg of TAMA per kg body weight in a volume of 2 ml/kg of Freon 113, the total excretion of fluoride in the urine averaged 69.6% of the administered dose in 7 days, ranging from 49.0% to 82.2%. During the first 2 days, an average of 35.5% and 19.9% of the administered fluoride was excreted, respectively. The fecal excretion of these same rats averaged 8.2% of the administered fluoride in 7 days. Six rats that were given intraperitoneally 2 ml/kg of Freon 113 excreted a daily average of 6.0 and 4.7 μg of fluoride during a period of 7 days in the urine and feces, respectively.

After administration of TAMA in Freon 113, the rats excreted a total of 72.0 ml of urine during the 7-day collection period. The total urine volume of each rat varied from 57.5 ml to 90.0 ml. The daily urine volume averaged from 4.8 ml to 16.3 ml. As reported in the section on AMOX, the control rats excreted an average of 10.6 ml to 13.2 ml of urine per day during a period of 5 days.

TABLE 31

THE AMOUNT OF FLUORIDE EXCRETED IN URINE AND FECES (PERCENT OF DOSE) AND URINE VOLUME OF RATS AFTER INTRAPERITONEAL INJECTION OF TAMA IN FREON 113

Weight of Rat (g)	Urinary Excretion Day After TAMA Administration 1	2	3	4	5	6	7	Total	Fecal Excretion
180	38.7	21.1	13.7	2.6	0.6	0.9	0.3	77.9	11.1
190	28.4	32.1	16.6	1.5	2.1	1.2	0.6	82.5	7.2
200	34.4	20.0	7.9	1.1	1.6	1.1	0.5	66.6	11.9
200	43.8	14.7	4.8	2.4	1.4	0.9	1.9	69.6	10.7
210	39.7	20.1	7.5	1.1	1.4	0.9	1.5	72.2	2.4
200	28.1	11.5	4.6	0.9	1.6	1.4	0.9	49.0	5.9
Average	35.5	19.9	9.1	1.6	1.5	1.1	0.9	69.6	8.2

Urine Volume, ml. Day After TAMA Administration 1	2	3	4	5	6	7	Total
13.0	12.0	9.0	13.0	16.0	18.0	9.0	90.0
4.5	8.0	7.0	11.0	16.0	14.0	6.5	67.0
3.5	9.0	7.0	10.0	15.0	6.0	7.0	57.5
1.5	15.0	7.0	9.0	16.0	7.0	8.0	63.5
0.5	17.0	10.0	18.0	18.0	8.0	10.0	81.5
5.5	9.0	8.0	9.0	17.0	16.0	8.0	72.5
4.8	11.7	8.0	11.7	16.3	11.5	8.1	72.0

SECTION IV

DISCUSSION

A. Toxicity of AMOX

The acute inhalation toxicity of AMOX appeared to be due to severe pulmonary irritation as evidenced by edema formation and extensive pulmonary hemorrhage. These effects led to death within 24 hr. Among the gaseous oxidizers investigated, nitrogen dioxide has been reported to be a respiratory irritant, causing pulmonary edema (ref 19) and pulmonary hemorrhage (ref 20).

The biological data previously available to us on AMOX indicated that rats weighing about 175 g died after exposure to 400 ppm for 10 min, and that no deaths occurred at 100 or 200 ppm (ref 21). This early observation was in agreement with the results obtained in the present investigation. The acute vapor toxicity of AMOX was quantitatively comparable to that of nitrogen dioxide. Gray et al (ref 19) reported the LC_{50} of nitrogen dioxide vapor for male albino rats to be 138 and 67 ppm after exposure of 30 min and 4 hr, respectively.

Intraperitoneal administration of AMOX caused strong local reaction. The abdominal cavity of the rats that died had undergone extensive denatured changes, especially of the peritoneal membrane and fat tissue around the kidneys. Both the abdominal and thoracic cavities contained various amounts of clear exudate. The weight of the lung significantly increased at 1 day, indicating the presence of pulmonary edema. The lung weight returned to the normal range of control rats at 7 days. The adrenals were significantly enlarged at 4 hr and 1 and 7 days. Administration of AMOX also caused hypotension in the pentobarbital sodium anesthetized rats.

The toxic symptoms after intraperitoneal administration were mainly abdominal distress and respiratory difficulty. Within a few hours, the animals showed weakness, depression, and died within 24 hr. The nature of the toxicity on the central nervous system was not fully investigated. After 7 days, the surviving animals appeared to be normal, but less active than the control rats.

AMOX undergoes decomposition in contact with tissue water. One of the possible breakdown products would be HF. The acute intraperitoneal toxicity of HF in male rats was found to be similar to that of AMOX. The rats died within a few hours, and clear exudate was accumulated in the abdominal cavity. The intraperitoneal LD_{50} of HF was between 20 and 25 mg/kg body weight, whereas that of AMOX was 51.6 mg/kg body weight.

There were both species and sex differences in the acute toxicity of AMOX. AMOX was more toxic to the mice than to the rats. The male rats were more resistant than the females.

B. Toxicity of TAMA

The acute inhalation toxicity of TAMA was not investigated due to its low vapor pressure. The intraperitoneal LD_{50} of TAMA for rats was greater than that of AMOX.

After intraperitoneal administration of TAMA there was severe abdominal distress and increased excitability. The rats showed signs of abdominal tenderness and painful distress. They reacted quickly to stimuli and attacked any unusual objects in their vicinity. Apparently, these were the result of strong local effects after TAMA had been injected into abdominal cavity. As in the case of AMOX, male rats were more resistant to TAMA than were the females.

The onset of toxic effects of TAMA was slower than that of AMOX. The rats after TAMA administration died slowly and showed weakness, depression and loss of appetite. The rats that survived usually lost weight and remained weak. Both AMOX and TAMA, and possibly their breakdown products, were quickly absorbed after intraperitoneal administration. Thus, the difference in the onset and course of toxic effects after intraperitoneal administration of AMOX and TAMA might be due to the different rates in the release of toxic product or products.

C. Absorption of AMOX and TAMA

After intraperitoneal administration, both AMOX and TAMA and possibly their breakdown products were quickly absorbed by rats. Total fluoride concentration of the blood increased rapidly, reached peak level about 1 hr and returned to the level of control animals after about 7 days. The blood fluoride was quickly concentrated in the various tissues investigated, presumably bound to the tissue proteins. The concentrations of fluoride in the liver, spleen, kidney, heart, lung, brain, muscle, and adrenals reached peak levels at 1 to 4 hr and returned to the normal ranges of control rats 7 days after administration of AMOX or TAMA.

The bones and teeth were known to contain high concentrations of fluoride and to combine readily with absorbed fluoride, as in endemic fluorosis areas or under conditions of abnormally high intake of dietary fluoride (ref 22). After single intraperitoneal administration of fluorine-containing AMOX or TAMA, the fluoride deposited gradually in both the femur and teeth and reached peak levels at about 1 to 7 days. The disappearance of fluoride was

slower in the femur and teeth than in the soft tissues. This relatively slow rate of movement of fluoride into and out of the femur and teeth may be attributable to the special affinity of this element for the probable apatite structure of the main mineral component of these tissues.

The bones and teeth have a remarkable capacity to store fluoride. The early clinical manifestations in livestock suffering from endemic or experimentally induced chronic fluorosis were lesions in bones, joints, and teeth (ref 22). When a saturation state of fluoride in these tissues was reached, the absorbed fluoride was then free to cause general toxic effects and death occurred. The acute toxic effects observed in the present investigation after administration of AMOX and TAMA were apparently not due to fluorosis.

The absorbed fluoride after intraperitoneal administration of AMOX or TAMA was quickly excreted in the urine. More than 50% of the administered fluoride was recovered in the urine during the first 2 days, and about two-thirds of the dose in 7 days. An additional 10% of the fluoride was excreted in the feces during the same period of 7 days. Many drugs and compounds are known to be excreted to the intestine through the biliary systems and others gain entry to the gastrointestinal tract directly through the wall. What portion of the fecal fluoride excreted through the biliary system and what portion crossed the gastrointestinal wall were not determined.

D. Fluoride in the Thyroid

The capacity of the thyroid to absorb and retain fluoride in domestic animals that had consumed water containing fluoride for an extended period has been reported (ref 22). It is of great interest to note the high affinity of fluoride for the thyroid following single intraperitoneal administration of AMOX and TAMA. The thyroid of these rats continued to take up fluoride from blood when concentrations of this element in the blood and other tissues were decreasing. At 7 days following administration, concentrations of fluoride in the thyroid were 16 times or more higher than those in the blood and various soft tissues which had returned to the normal concentrations of control rats. Fluoride concentration of the thyroid returned to normal range after about 4 to 6 weeks.

A few studies on the interaction between fluoride and thyroid activity have been reported and the results are confusing. Basal metabolism in normal animals decreased following oral or parenteral administration of sodium fluoride (ref 23) or parenteral administration of hydrofluoric acid (ref 24). However, Phillips and coworkers (ref 25) found that sodium fluoride had no effect on the basal metabolism of normal animals, but increased slightly the metabolic rate of hyperthyroidal animals treated with desiccated thyroid. The latter investigators (ref 26) also reported that chronic feeding of sodium fluoride made the nontoxic levels of desiccated thyroid distinctly toxic in the growing chick. Concentration of fluoride in the thyroid was not determined by these investigators. The observation of high concentrations of

fluoride in the thyroid found in the present studies may be due to the fluoride entering the thyroxine molecule rather than being bound simply to the thyroid protein.

SECTION V

CONCLUSIONS

A. AMOX

1. The LC_{50} of AMOX for the adult male rats at the end of 7 days after acute exposure for 4 hr, 60, 30, and 15 min was 24.2 ppm, 87 to 104 ppm, 119 to 149 ppm, and 202 to 240 ppm, respectively. The acute inhalation toxicity of AMOX was greater for mice. The LC_{50} for adult male mice at the end of 7 days after a single exposure of 4 hr was 17.5 ppm.

Death after inhalation appeared to be due to respiratory irritation resulting in pulmonary edema and hemorrhage. Other toxic effects involving the central nervous system were excitation and depression, followed by convulsions. The animals that survived acute exposure showed severe depression, loss of muscle tone and weakness. Most of the deaths occurred within 24 hr after beginning of the exposure. No animal died after 7 days. The lungs of surviving animals showed various stages of regeneration at intervals after exposure.

2. The intraperitoneal LD_{50} of AMOX for adult female and male rats at the end of 7 days was 10.8 ml (38.4 mg) and 14.5 ml (51.6 mg) per kg body weight, respectively. The AMOX after intraperitoneal administration was also more toxic to mice. The intraperitoneal LD_{50} for female and male mice at the end of 7 days was 8.3 ml (29.5 mg) and 9.2 ml (32.1 mg) per kg body weight, respectively.

The toxic symptoms after intraperitoneal administration of AMOX included abdominal distress, respiratory difficulty, weakness, and severe depression. Most of the deaths at the various dose levels occurred during the first day after administration. There were local denatured changes and accumulation of clear exudate in the abdominal cavity.

3. After intraperitoneal administration of AMOX to rats, there was edema formation in the lung and enlargement of the adrenals. AMOX administration also caused a drop in blood pressure of pentobarbital sodium anesthetized rats.

4. Intraperitoneal administration of AMOX resulted in hemoconcentration due to shift of body fluids into the abdominal cavity. The red blood cell count, hematocrit and hemoglobin concentration increased quickly. After 1 day, the red blood cell count, hematocrit and hemoglobin concentration decreased, whereas both the mean corpuscular volume and the mean corpuscular hemoglobin increased. Both transaminase levels of rats during the first day after AMOX administration were higher than the levels of the control rats.

5. The AMOX and/or its breakdown products were quickly absorbed from the abdominal cavity of rats. Total fluoride concentration in blood reached peak level about 1 hr after administration and remained slightly higher than the level of control rats at the end of 7 days. Higher concentrations of fluoride were found in the liver, spleen, kidney, heart and lung, whereas those in the brain, muscle and adrenals were lower. Fluoride concentrations in these tissues were lower than in the blood of the same animal. The tissue fluoride reached peak levels at about 1 to 4 hr and returned to the normal ranges of control rats at 7 days.

The total fluoride concentration of the thyroid increased quickly after AMOX administration and reached a peak level at the seventh day at which time the concentration was 17 times higher than that of blood. The concentration of fluoride in the thyroid returned to the normal range of the control rats about 4 to 6 weeks after AMOX administration.

As expected, both the femur and teeth of control rats contained high concentrations of total fluoride. After AMOX administration, concentrations of fluoride in femur and teeth increased and reached peak levels at about 1 to 7 days. The total fluoride disappeared gradually from the femur and teeth, and concentrations in these two tissues returned to the normal levels of the control rats about 2 weeks after administration.

The majority of the administered fluoride following AMOX was excreted in the urine. An average of 61.1% of the administered fluoride was excreted in 7 days, with 33.6 and 20.2% of the dose excreted during the first 2 days, respectively. These rats excreted a total of 9.7% of the administered fluoride in the feces in 7 days.

B. TAMA

1. The intraperitoneal LD_{50} of TAMA for adult female and male rats at the end of 7 days was 94 mg and 140 mg per kg body weight, respectively. TAMA was less toxic after oral administration. The oral LD_{50} for both female and male rats was greater than 200 mg/kg body weight.

The toxic symptoms after intraperitoneal administration of TAMA were severe abdominal distress, increased excitability followed by weakness and depression. The animals lost appetite and died slowly in various distress positions. The rats that survived the observation period of 7 days lost weight and remained weak.

2. Intraperitoneal administration of TAMA had no effect on blood pressure of the pentobarbital sodium anesthetized rats. The adrenals of these rats were enlarged at 1 day.

3. Administration of either TAMA in Freon 113 or Freon 113 alone resulted in accumulation of clear exudate in the abdominal cavity. This shift of body fluids accounted for the early increases in red blood cell count, hematocrit, and hemoglobin concentration. No change in hematology or functional tests was attributed to the TAMA administration.

4. As in the case of AMOX, the TAMA and/or its breakdown products were quickly absorbed by rats. After intraperitoneal administration, concentration of fluoride in the blood increased rapidly and reached a peak level at about 1 hr. At 7 days, the total blood fluoride returned to the normal range of control rats. Higher concentrations of fluoride were also found in the liver, spleen, kidney, heart, and lung, whereas the brain, muscle, and adrenals contained less. Fluoride concentrations in these tissues did not exceed that of the blood in the same rats. They reached peak levels in 1 to 4 hr and decreased to the normal ranges of the control rats in 7 days.

Concentration of fluoride in the thyroid increased rapidly after TAMA administration, reached peak level at approximately 24 hr, and returned to control level at about 6 weeks. The fluoride concentration of the thyroid was 16 times higher than that of the blood 7 days after TAMA administration.

Fluoride concentrations of the femur and teeth increased after TAMA administrations and reached peak levels in 1 to 7 days. Concentrations of fluoride in these two tissues returned to control levels at 2 weeks.

After TAMA administration, most of the administered fluoride was excreted in the urine. These rats excreted a total of 69.6% of the dose in 7 days with an average of 35.5% and 19.9% of the dose during the first 2 days, respectively. A total of 8.2% of the administered fluoride was excreted in the feces during the period of 7 days.

SECTION VI

SUGGESTIONS FOR FUTURE STUDIES

Some useful information has been obtained in the present studies on AMOX and TAMA. The following areas of research are of particular interest for future investigation.

A. Absorption, Distribution, and Excretion Pattern of AMOX after Inhalation

The absorption, distribution and excretion patterns of fluoride after intraperitoneal administration of AMOX have been thoroughly studied. The

AMOX is in gaseous state at room temperature and under atmospheric pressure. The danger for industrial production and military handling of this material lies in the possibliity of acute exposure to the vapor. The accumulation of information on the absorption, distribution, and excretion pattern after inhalation of this gas would be of real significance.

B. Interaction of AMOX and Thyroid Activity

The effects of ingestion and injection of fluoride on thyroid activity have been contradictory. High concentrations of fluoride have been found in the thyroid after administration of AMOX. Further studies on the effects of chronic administration of or exposure to AMOX on the thyroid activity, to study the fluorine-iodine interaction, and to investigate the possible formation of fluorinated thyroxine are needed.

C. Mechanism of AMOX Toxicity

Both the inhalation and intraperitoneal toxicities of AMOX have been investigated. AMOX produced pulmonary edema, depressed blood pressure and caused death quickly. The information of the biochemical changes following exposure to AMOX is of value for diagnostic and therapeutic purposes.

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Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Midwest Research Institute 425 Volker Boulevard Kansas City, Missouri 64110	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP N/A

3. REPORT TITLE

METABOLISM & PHARMACOLOGY OF FLUORINE-CONTAINING COMPOUNDS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final report February 1965 - June 1966

5. AUTHOR(S) (Last name, first name, initial)

Lee, Cheng-Chun

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
July 1966	73	26

8a. CONTRACT OR GRANT NO. AF 33(615)-2472 b. PROJECT NO. 6302 c. Task no. 630202 d.	9a. ORIGINATOR'S REPORT NUMBER(S) 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) AMRL-TR-66-110

10. AVAILABILITY/LIMITATION NOTICES This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Aerospace Medical Research Laboratories (MRBTT), Wright-Patterson Air Force Base, Ohio

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY Aerospace Medical Research Laboratories Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, Ohio

13. ABSTRACT

Acute inhalation, intraperitoneal and/or oral toxicities of AMOX and TAMA were determined in rats and mice. IP absorption was studied in rats.

AMOX produced strong irritation resulting in pulmonary edema and hemorrhage after inhalation; and local denatured changes and accumulation of clear exudate after intraperitoneal injection. TAMA was less toxic on the weight basis.

Both AMOX and TAMA were quickly absorbed after IP injection. Fluoride increased quickly in the blood and concentrated in various tissues especially in the thyroid, bone and teeth. Most of the administred fluroide was excreted in the urine and a considerable amount was also recovered in the feces.

DD FORM 1 JAN 64 1473

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14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
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AMOX and TAMA						
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Fluoride						
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